ANNUAL REPORT

MENTAL HEALTH INTRAMURAL RESEARCH PROGRAM - Division of Clinical and Behavioral Research, Division of Biological and Biochemical Research, and Division of Special Mental Health Research

NATIONAL INSTITUTE OF MENTAL HEALTH

October 1, 1981 - September 30, 1982

VOLUME I

Summary Statements

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of the
DIRECTOR, MENTAL HEALTH INTRAMURAL RESEARCH PROGRAM
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Frederick K. Goodwin, M.D.

This will be my first annual report as Director of the Mental Health Intramural Research Program. In the tradition of my predecessor, Dr. John Eberhart, I shall use a personal rather than a formal style in summarizing the events of the past year and commenting on their impact on the program from my perspective. All in all, I should say it has been a good year. Exciting, difficult at times, demanding, but always rewarding. Our research flourished in spite of the disruptions caused by the change in leadership, staff dislocations which resulted from a reduction-in-force, the departure of senior scientist-administrators, some of whom headed major research components, and a continuing space shuffle occasioned by the opening of the ACRF.

Evidence of the continued vigor of our program abounds. Dr. Louis Sokoloff was given the Lasker Award. Dr. Erminio Costa was elected to the National Academy of Sciences. Drs. David Pickar, Martin Cohen, Robert Cohen and Dieter Naber captured the A.E. Bennett Award. The Stanley R. Dean Award went to Dr. Richard Wyatt, and Drs. Sokoloff and Giulio Cantoni were elected Fellows of the American Academy of Arts and Sciences. Dr. Sokoloff and I received Presidential Rank Awards for Meritorious Executives. Dr. Marian Yarrow received the University of Minnesota's Outstanding Achievement Award, and Dr. Irwin Kopin a Burroughs Wellcome Fund visiting professorship at the University of Arizona.

The continuing high quality of IRP science was also recently recognized when nine of our scientists made the Current Contents list of the 1,000 most frequently cited scientists in the world over the past 15 years (that is, the top one-tenth of 1 percent of all published scientists). That ranks the Mental Health Intramural Program near the top cited institutions worldwide, and in the top third among its sister intramural programs of the NIH Institutes. Further, there is viable evidence that our work has won greater recognition and appreciation among the public, Congress and with the Administration as well as the scientific community.

My gratitude goes out to the lab chiefs and other senior staff members who helped me through this challenging period. I am particularly indebted to John Eberhart, whose 20-year legacy as Director serves me daily as a model of stable and wise leadership. Also to Dr. Bob Cohen, who as Deputy Director displayed a special feel for the complex clinical side of the program. John Eberhart left the Institute last year to become a special advisor to the NIH Director. Bob Cohen, who filled in as Director during the interim, is now Director of Psychotherapy at Chestnut Lodge. In addition to a vigorously flourishing program, John and Bob left me a precious legacy - the respect and affection of the NIH Community. Although awed by the task, I am committed to sustaining and nourishing their legacy.

A towering presence providing vital continuity during the transition was Hazel Rea, the best administrator I have experienced in my 18 years in the Government. Profoundly dedicated to IRP, she knows its people and its substance inside and out. This in-depth knowledge, combined with incredibly good judgment about people, gives her the capacity to deal with them firmly but with respect and sensitivity. As close to indispensable as anyone could be, she deserves singular credit for the successful transition.

In a different, but equally important way, Drs. Irv Kopin and Ed Evarts made an indispensable contribution to the success of this transition year. In essence, Irv, Ed, Hazel and I functioned as a leadership team with all major program decisions flowing out of a vigorous and rewarding conjoint process. To this process Ed and Irv brought their accumulated wisdom about the nature of science; their deep affection for, and commitment to this scientific enterprise and its people would be difficult to overstate. I have learned and continue to learn a great deal from them.

Also meriting special mention was the remarkable degree of support and stable leadership provided during this period by Dr. Herbert Pardes and his staff at NIMH headquarters, by Dr. William Mayer and coworkers at ADAMHA, and by Dr. Edward Brandt and his colleagues at the PHS and Departmental levels. Dr. Pardes' serious commitment to substance and quality has been especially heartening to the IRP. He initiated a series of weekly halfday visits to IRP labs for in-depth briefings, substantially enhancing his effectiveness as a convincingly knowledgeable advocate for the Institute's research programs, both extramural and intramural. Another positive factor in this transition period has been the continuing support and cooperation of the National Institutes of Health. The Scientific Directors welcomed me into their group and I have learned a great deal from my interaction with them. Drs. Ed Rall and Phil Chen, who have each served as Acting Deputy Director for Science during this year, were ever generous with their wise counsel and help. I am glad to say we continue to enjoy our status, established in past years, as a member of the National Institutes of Health family.

Before moving on to another issue I should like to acknowledge the wisdom and strength we draw from our Board of Scientific Counselors. They work hard to help us sort out complex problems and we benefit greatly from their advice. The following Laboratories were reviewed during this reporting period: In October, 1981, the Laboratories of Cerebral Metabolism (Dr. Louis Sokoloff, Chief), Neurobiology (Dr. Ichiji Tasaki, Chief), and Neurophysiology (Dr. Edward Evarts, Chief); in March 1982, the Laboratories of Neuropsychology (Dr. Mortimer Mishkin, Chief) and Psychology and Psychopathology (Dr. Allan Mirsky, Chief). The following changes were made in Board Membership: Board members whose four-year terms ended in June 1982 were Drs. Morton Reiser, who also served as Board Chairperson, and Solomon Snyder. Dr. J. Allan Hobson continues as Board member and took over as Board Chairperson upon Dr. Reiser's departure. New Board members appointed during this period are Drs. Norman Garmezy, Anita Hendrickson, Brenda Milner, and Robert Moore.

We are at once saddened and proud to note the departure of three of our major clinical investigators over the past year. Drs. William Bunney, Chris Gillin and Monte Buchsbaum have moved on to well-deserved leadership positions on the West Coast. Dr. Bunney, who was chief of the

Biological Psychiatry Branch, ended a distinguished career of 24 years with IRP to become Director of Psychiatry at the University of California at Irvine. Dr. Buchsbaum, who came to IRP in 1966, has now joined Dr. Bunney at Irvine to continue some of the brain imaging studies he had begun here. Brain imaging will continue to be a major program focus of the IRP, but we have lost an institutional treasure in Dr. Buchsbaum's skill at integrating findings and interpreting them to the laity. Dr. Gillin, an outstanding biological psychiatrist, has gone to the University of California at San Diego, where he will continue his sleep studies on a larger scale than our limited resources would have allowed.

As much as we regret the exodus of these fine investigators and feel the loss of their contributions to the program, we recognize that changes can have positive effects, both for the IRP and for the research community at large. They allow growing room for our talented young people and enrich other programs with well-winnowed mentors who have proven themselves in the unique environment of the IRP. It is a fair exchange. What's more, the process can come full circle, and it has this year. Dr. Seymour Kety, the IRP's first Director, who has been at Harvard since 1968, is coming back! Now the leading statesman of our field, he is still an active and productive investigator, whose recent genetic studies of schizophrenia stand as a landmark. Currently serving as a part-time consultant, Dr. Kety, is the father of the modern era of biological psychiatry and its integration with the neurosciences. I count my recruitment of Dr. Kety to the fulltime post of IRP Associate Director for Basic Research (next September) as my most gratifying moment so far this year.

Establishment of that position is part of a broader reorganization of IRP begun over the past year which is best understood in the context of the personnel changes. Vacancies were created in two of the three Division Director and two key Branch Chief positions when Drs. Eberhart, Cohen and Bunney left and I became Director. It was an opportune time for an updating of the organizational structure. The purported distinction between "clinical" and "basic" research embodied in the Divisional structure was not borne out by reality. While it made some sense in light of the professional qualifications of the outgoing leadership team, it did not capitalize on one of IRP's great strengths - its ability to build bridges between the laboratory and the patient, to pursue clinical and basic research in parallel. In practice, this vital integration takes form less in structured programs than in the kind of scientists we develop. A research psychiatrist may require a broad range of laboratory and clinical skills to translate the explosion in basic knowledge about pharmacology and neurobiology into new drug treatments that address pressing human needs. This mandates that high quality basic research go on in "clinical" branches and vice versa. It also requires increasing collaboration across laboratories and divisions. One example is the receptor mapping methodology pioneered by Drs. Candace Pert and Miles Herkenham, a joint venture of the Laboratory of Neurophysiology and the Biological Psychiatry Branch. Others include Dr. Louis Sokoloff's interest in the clinical applications of Positron Emission Tomography and Dr. Edward Evarts' extension of techniques developed for basic research on the neurophysiology and neuroanatomy of movement to the motor problems seen in Parkinson's Disease and other clinical conditions.

It is this perception of the actual workings of the IRP research enterprise, along with the personnel changes, which led to a decision to abolish the divisional structure. Administering the program as a single entity under a leadership team comprised of Associate Directors each of whom has staff responsibilities for discrete areas, has a number of advantages, not the least of which is greater flexibility in this deployment of resources. A dynamic enterprise such as ours demands such flexibility. As noted earlier, Dr. Edward Evarts, Chief of the Laboratory of Neurophysiology, served with distinction in an acting capacity as Associate Director for Basic Research during the bulk of the transition year. Following the timetable he had set for himself initially, Ed has returned to fulltime research and Lab Chief responsibilities but continues as a trusted advisor on an informal basis. Dr. Irwin Kopin continues to serve as Associate Director for Clinical Research, while Dr. Richard Wyatt serves as Associate Director for Intramural Research at Saint Elizabeths Hospital and Mrs. Hazel Rea as Associate Director for Program Management. Drs. Wyatt and Kopin continue to have responsibilities as Laboratory chiefs.

To strengthen the coordination of our clinical research programs and their integration with other Institute programs, I early on selected Dr. Michael Ebert to be IRP Clinical Director. Reflecting the high esteem in which he is held by his fellow clinical directors at NIH, Dr. Ebert has also been appointed chairman of the NIH Medical Board. His efforts have been instrumental in preparing our program and the Clinical Center at large for reviews by the Joint Commission on Accreditation of Hospitals and the American Hospital Association over the past year. Dr. Bill Potter serves as my special assistant, while continuing his research in clinical pharmacology.

Pending official approval of the reorganization plan, the administrative functions of the two divisions on campus will be combined; a separate administrative office will continue to be maintained at Saint Elizabeths. The new system is working well and I am especially gratified by my interactions with the Associate Directors; in my view this team leadership structure reduces the likelihood of major policy mistakes while preserving the flexibility and vigor necessary for innovation.

A spate of other new appointments followed from the need to provide fresh leadership and focus for my former Branch (Clinical Psychobiology) and Dr. Bunney's Biological Psychiatry Branch. Sections of these branches devoted to neuroscience studies were brought together to create a new Clinical Neuroscience Branch headed by Dr. Steven Paul, an exceptionally gifted young investigator, who possesses an impressive combination of laboratory and clinical skills. The new branch consolidates resources and catalyzes collaborations among such outstanding young scientists as Drs. Candace Pert, John Tallman and David Pickar, who serve as section chiefs.

The Clinical Psychobiology Branch (CPB) is now sharpening its focus on circadian rhythms in affective disorders and related clinical pharmacology and sleep studies under its new acting chief Dr. Thomas Wehr. Laboratory studies of circadian and sleep physiology will receive new emphasis as Dr. Wallace Mendelson moves over from Dr. Wyatt's lab to the CPB.

Under Acting Chief Dr. Robert Post, the Biological Psychiatry Branch

(BPB) is evolving a new structure, incorporating into its well-functioning clinical operation a laboratory with the capacity to measure neurotransmitters and their metabolites under Dr. David Jimerson, who is transferring over from the Laboratory of Clinical Science. Also, Dr. Philip Gold, formerly in my former Branch, is setting up a Section on Clinical Neuroendocrinology in BPB.

A new Laboratory of Cell Biology has been established under Dr. Michael Brownstein, one of the leading young neuroscientists in the world. Additional resources will allow an expanded effort in studies of the anatomy, physiology, biochemistry, pharmacology and development of peptidergic neurons, and for the application of molecular genetics techniques to these problems. This new lab was spawned from the Laboratory of Clinical Science, which in turn, is creating a new Section on Child Psychiatry under Dr. Judith Rapoport, formerly with the BPB under Dr. Bunney, and a Section on Analytical Biochemistry headed by Dr. Sanford Markey.

NIMH was one of the first Institutes to begin occupying space within the Ambulatory Care Research Facility (ACRF), the new outpatient wing of the Clinical Center, which opened this past year. As our new Associate Clinical Director for Outpatient Studies, Dr. Rex Cowdry has overseen a three-fold increase in the number of outpatient visits during this period, reflecting a significant expansion of our clinical research capacity. The clinical programs have taken advantage of the new facilities by mounting new outpatient research in such areas as phobia, panic-anxiety, obsessive-compulsive disorder, borderline personality disorders, childhood mental illness, and "well state" studies of manic depressive patients and their families, under the leadership of Dr. Elliot Gershon. The integration of inpatient and outpatient studies is also leading to briefer hospital stays overall.

Another potentially valuable resource for outpatient studies is the Mental Health Study Center in Adelphi, Maryland. IRP has now inherited part of this program orphaned by the conversion to State block grants of the gutted NIMH Services Program. Its clinical infant development project has been placed administratively within Dr. Allan Mirsky's Laboratory of Psychology and Psychopathology. We are in the process of helping Dr. Stanley Greenspan and his staff retool from their former mission, services research and demonstration, into a genuine clinical research organization, building on Dr. Greenspan's psychodynamic studies of infants, which won him the Strecker Award this past year. We also hope to take advantage of the Center's strong community ties and established catchment of patients.

Also housed within Dr. Mirsky's lab is our growing brain imaging program. The IRP will be purchasing a Positron Emission Tomography (PET) scanner next year with twice the resolving power of existing equipment at NIH. The FY '83 budget also calls for three new staff positions tagged to the PET operation. This signals a major commitment by the Administration to mental health related PET research and testifies to the success of Dr. Pardes' efforts to communicate the promise of this new technology. As we continue to collaborate with Dr. Buchsbaum and finish PET and EEG mapping projects he started, we are also carefully searching for a successor to provide new leadership for future imaging studies. For its part, NIH has brought on board Dr. Steve Larson, a leading PET investigator, to head

its Nuclear Medicine Department. We look forward to working closely with him. The PET studies will also benefit from safer, short half-life radioisotopes in a few years when a cyclotron is expected to be in operation on the NIH campus.

A major focus of my efforts over the past year has been to improve communications both within the IRP and with our various external "publics". Toward this end, I initiated and devoted considerable time to a series of visits to the various labs which make up the Program. These visits afforded me a feel for the particular strengths and problems of each unit and gave me the opportunity for in-depth discussions of work in progress in many cases.

The Associate Directors accompanied me on some of my day-long visits to IRP labs housed in the William A. White Building at Saint Elizabeths Hospital, where we immersed ourselves in the detail of the science being conducted there. We were struck by how remarkably successful that program is, despite its isolation and recurrent problems with the physical plant and patient care staffing. Since we have serious concerns about the long-term viability of that program in that setting, we were encouraged recently to learn that planning may soon begin for a new building on the NIH campus which would house our units now at Saint Elizabeths along with components of NIH and Food and Drug Administration. In the meantime, we will continue short-term renovations of the WAW Building at St. E's and work out resource allocation issues with the Hospital to assure adequate personnel on the research wards.

A major new effort at internal communications was begun this year with the inauguration of an IRP-wide Director's Conference Series. These morning-long symposia are intended to provide a regular opportunity for IRP scientists to facilitate increased interaction among the labs. My hope is that as investigators working on related problems become more knowledgeable about each other's work, that approaches developed in one lab may find more rapid applications in another; and that the quality of research will benefit from interdisciplinary cross-fertilization and collaboration. The series has initially emphasized topics at the intersection of basic neuroscience and clinical psychobiology. During 1982, the seven member conference committee, chaired by Dr. Carl Merril, has put together presentations on the following subjects: molecular genetics, electrophysiological approaches to the pharmacology of neurotransmitter function; biological studies in schizophrenia, and imaging of brain metabolism and physiology, and a reevaluation of the role of neurotransmitter amines in affective disorders. Each of four or five speakers presents his/her work with a short discussion period at the end. The first two conferences played to standing room only crowds in the cramped Building 36 conference room; the second two nearly filled the ACRF amphitheater, an ideal setting for the event. In future conferences we intend to expand the time available for open discussion. Each conference is followed by senior staff and lab chiefs' meetings later in the afternoon, a convenient arrangement for scientists attending from our far-flung labs at Poolesville, Saint Elizabeths and across campus.

To help keep IRP personnel abreast of workshops and speakers sponsored by the various labs, we have also begun issuing a monthly calendar of events.

Having the Director's office in Building 36 for the first year was fortunate in that it gave me an opportunity to get better acquainted with the research staff located there. I decided, however, to move my office back to Building 10, where the Director's office was originally, so that I might be in closer proximity to the bulk of the program which still resides in that building. In the interest of fostering communication between the geographically separate parts of the program, we will continue to hold lab chief and senior staff meetings and various other functions in the Building 36 conference room.

The other major communications thrust of the past year has been in the area of public awareness of research. In response to increased public interest in our work, we have taken a number of steps to educate the media and other interested groups more systematically. I have long felt that scientists have an obligation to explain and translate their work to professionals outside their specialty and to the lay public. Our past failure to devote sufficient care to these efforts can be reflected in public misunderstandings, damaging to research, about such issues as the use of animals in research and the protection of human subjects. Dr. Pardes has been especially sensitive to the needs for vigorous, careful public education and efforts in this regard directly inspired a six-part series on the brain in the Washington Post last August which featured a large number of our scientists. It was quite well done and elicited much favorable comment. Admittedly, taking time to talk to reporters, and staging action for photographers and TV crews, can be disruptive to a research organization; some of our labs have been literally inundated with press inquiries over the past year. To help take the pressure off individual scientists and to improve the quality and balance of the information provided, I have restored a public information function within the IRP after an eight year hiatus. This role is filled by Jules Asher, an NIMH science writer who fortuitously "bumped" into the program in last winter's RIF. His responsibility as "broker" between the scientists and the media is closely coordinated with that of Dr. Julius Segal and his information staff at NIMH headquarters.

A special committee of senior intramural staff members prepared a report this past year on issues raised by the DHHS Standards of Conduct and their relationship to IRP. This report, authored by Dr. Theodore Colburn, is especially timely in light of the Administration's efforts to encourage more flexible cooperation between Government and the private sector. It points up a need to re-examine policies on outside activities for scientists in the light of the new initiatives of this Administration. The report has been forwarded to Drs. Pardes and Mayer where it is part of a continuing discussion which will form the basis for policy guidance.

Finally, the continued health and vigor of our intramural program is inexorably linked to the vitality of the research enterprise throughout the country, an enterprise heavily dependent on our Extramural grant programs. We are deeply aware of the responsibility that derives from our position as the largest mental health research program in the world and from our location here in the shadow of the nation's capital. We are committed to using our credibility as a center of excellence on behalf of the entire mental health research community.

ANNUAL REPORT OF THE DIVISION OF SPECIAL MENTAL HEALTH RESEARCH
NATIONAL INSTITUTE OF MENTAL HEALTH
October 1, 1981 to September 30, 1982

As I write this report I feel considerably more comfortable about the state of ADAMHA, NIMH and the Intramural Research Program than at any time during the last year. Drs. Herbert Pardes and William Mayer, the Directors of NIMH and ADAMHA respectively, have added a welcome stability in leadership. Their strong support and interest in research coupled with their friendly and competent styles have given the Program a steadiness and warmth hard to find in most government institutions.

The recent past, however, has been fraught with considerable turmoil. Dr. John Eberhart, the Director of the Intramural Research Program for 20 years, announced he would step down in June 1981, and Dr. Robert Cohen, the Deputy Director announced his retirement, effective December 1981. Given the hiring freezes, it took an inordinate amount of time for the search committee to be established and conclude its work. Concern surrounding the vacancies mounted for almost 18 months. Anxieties over what changes the new Director might make, in what had been an extremely stable program under Drs. Eberhart and Cohen, built. Concern continued as the newly appointed Director, Dr. Frederick Goodwin, reorganizationed the Program. At this writing, though, the anxieties appear to be subsiding. Most of the Intramural Program's large and diverse staff support Dr. Goodwin's programatic, administrative and scientific changes and his long association with the Program provides continuity and stability as the changes take place. The Program has a new excitement to it which is greatly appreciated.

In addition to Drs. Eberhart and Cohen, we regret seeing Drs. William Bunney and J. Christian Gillin leave the NIMH. Dr. Bunney has been a good friend to the Division of Special Mental Health Research, and we wish him much success in his chairmanship of psychiatry at the University of California at Irvine. Dr. J. Christian Gillin, who has been the Deputy Director of the Adult Psychiatry Branch, has taken a professorship at the University of California at San Diego, and we wish him, also, much success. The friendship, advice and leadership of these two outstanding scientists will be sorely missed.

We are, unfortunately, still feeling the drastic cutbacks in the ADAMHA budget for 1982, including the reduction in force (RIF). The RIF caused many to lose their jobs or be displaced into positions for which they were poorly qualified. Because of terminations in programs in the Parklawn Building, a number of individuals, through no fault of their own and who had excellent service records, were downgraded into inappropriate positions or lost their positions entirely. While many of these individuals made the best they could out of a bad situation, others felt they were dealt with unfairly and were soured by the experience.

What appears to be the most serious and lingering problem, however, is that the Division of Special Mental Health Research's relationship with Saint Elizabeths Hospital has deteriorated in the last year. The hospital has had severe budget cutbacks and consequently has given us even poorer service than in the past. Maintenance services from the hospital are at an all-time low. Because of overage positions at the hospital, we have been unable to appoint a chief nurse or reshape our nursing personnel to place more professional nurses on our wards. Further, our ability to replace nursing staff, supplied by the hospital, has been reduced considerably. If there are further reductions, we will have to close one of our wards. Perhaps within the year, the hospital will become a corporation. If this change

transpires, our dealings with the new administration will have to be made more formal than they have been in the past.

Thus, while the Division of Special Mental Health Research continues to face uncertainties, I am relieved to report that the tenor of the Intramural Research Program is upbeat. Most rewardingly, the scientific excitement and accomplishments in our Division remain high. During the last year we have had 16 guest workers from six countries. We also have had 22 Visiting Fellows from seven countries. We especially enjoyed having Drs. Ann-Zhong and Tang, from the Peoples' Republic of China working with us. We have invited over 100 speakers and published over 140 papers. Dr. Erminio Costa was elected into the National Academy of Sciences and I was awarded the Stanley R. Dean Award for outstanding research in schizophrenia.

Dr. Erminio Costa's Laboratory of Preclinical Pharmacology has had a very active and productive year, continuing work that began during the last few years. The major thrust of the laboratory has been to examine the function of two or more putative neuromodulators co-existing in the same neuron. In order to examine the reason for the existence of these substances co-existing in the same neuron, the laboratory has begun to view the synapse in a more complex manner then previously. Earlier notions viewed the synapse as a gap between neuron A and neuron B, with a neurotransmitter crossing the gap: the neurotransmitter being released at A with there being a receptor for it on neuron B. Today, the receiving neuron (B) is seen as having (1) a recognition site that receives the neurotransmitter, (2) a coupler system which transmits the information from the recognition site to, (3) a transducer that transforms the chemical signal into an active process. The active process can be either a specific ion flux (or gate opening) or enzyme activition (second messenger formation). The primary models the laboratory has used are the GABA snyapse where there are recognition sites for benzodiazepine with a special protein, GABA-modulin, which can be phosphorylated as an intermediary coupler, acetylcholine and the opiate recognition sites in the adrenal medulla, and serotonin and imipramine (an antidepressant) sites that are close together on specific neurons in the brain.

The Adult Psychiatry Branch's (formerly the Laboratory of Clinical Psychopharmacology) studies on schizophrenia advanced considerably during the last year. The Division was fortunate enough to receive a CT scanner that had been used by the National Cancer Institute in their Baltimore Facility. We are making this available not only for research purposes to the Divison, but to the hospital as well. Led by Dr. Daniel Weinberger's efforts using computed tomography (CT) scans, the Branch's ability to discriminate between two forms of schizophrenia has been refined. One form of schizophrenia appears to be associated with enlarged cerebral ventricles, the other with normal sized ventricles. These patients differ in their ability to respond to neuroleptic drugs, in their premorbid history and in their response to agents which affect the dopaminergic system. Also, Dr. Janice Stevens has advanced our knowledge of a possible viral etiology in schizophrenia by producing evidence that cytomegalovirus seems to be increased in the brains of patients with schizophrenia.

Structurally, we have completed more renovation of the building. We have installed a tissue culture laboratory for Dr. Joan Schwartz that is fully operational and is producing extremely interesting research. The first floor conference room was completed also and provides an excellent facility, seating sixty visitors.

Dr. Llewellyn B. Bigelow, who has been our Clinical Director for the William A. White Division, and Mr. Carl Pergler, ably shepherded our Division through the accreditation

process and Saint Elizabeths Hospital has now received full accreditation for two years. Largely due to Dr. Bigelow's and Mr. Pergler's efforts, the Joint Commission on the Accreditation of the Hospital found very few problems relating to safety or medical records in our building.

Mr. Paul Byrne, who has been with the Division almost since its inception, moved to Dr. Tasaki's laboratory on the main campus. Mr. Byrne had been a machinist with this Division and his skills and creativity were greatly appreciated. We know, however, that he will continue to grow in his new position and wish him well. He has been replaced by Mr. Ken Killian. Mr. David Bauler, the photographer, and Ms. Debbie Behun, the graphic artist, have been replaced by Mr. Leroy Sunberg and Mr. Avrum Ashery.

In closing, I would like to, again, note how much we appreciate the help of the administrators of ADAMHA, NIMH, and the Intramural Program, including Mrs. Hazel Rea for helping us get our work done. Mrs. Rea is always looking out for our interests and during her absence, due to illness, she was greatly missed. Finally, as I look back over the last year, while some periods felt a bit rocky, I see many of the bumps being smoothed. While clearly it is difficult to replace Drs. Eberhart and Cohen who had been so important in beginning and directing the Program, Dr. Goodwin's long association with the Program, as well as with NIMH, and his close relationship with Drs. Pardes and Mayer, have allowed this transition period to be much smoother than it might otherwise have been. I am looking forward to calmer seas in the year ahead.

ANNUAL REPORT OF THE BIOLOGICAL PSYCHIATRY BRANCH
National Institute of Mental Health
October 1, 1981 - September 30, 1982

Robert M. Post, M.D., Acting Chief

During the past year the Branch has continued to be productive and creative while evolving into a new structure. The current annual report is based on the old structure which existed during part of this transition period and includes the eight Sections or Units of the Biological Psychiatry Branch as originally constituted. However, Dr. Tallman and the Section on Biochemistry and Pharmacology will move to support the basic research laboratory of the Clinical Neuroscience Branch. Dr. Agu Pert from that group will stay in the Biological Psychiatry Branch and head up a group studying the interphase between behavioral pharmacology and neurotransmitter and peptide mechanisms underlying alterations in behavior observed in a variety of clinically relevant animal model systems. The Section on Neuropsychopharmacology will form the major clinical unit in the new Neuroscience Branch and will be led by Dr. David Pickar, formerly the Chief of the Unit on Studies of Drug Abuse. The Unit on Sleep Studies will be integrated into the new Laboratory of Chronobiology. The Unit on Childhood Mental Illness will have bed space and administrative direction from the Laboratory of Clinical Science.

The Clinical Research Unit of the Section on Psychobiology, located on 3-West, will become the core clinical research facility of the new Branch. Dr. T. W. Uhde will lead the major clinical research initiatives on the Unit in the studies of both mood and anxiety disorders. In addition to his work on affective illness, he has initiated a series of inpatient and outpatient studies in a new clinical population of patients with phobic-anxious and panic-anxious disorders. Dr. P. W. Gold, formerly with the Clinical Psychobiology Branch, and Dr. D. R. Rubinow will continue and expand the major neuroendocrinological and neuropeptide initiatives in the Branch. This will involve both inpatient and outpatient programs and close collaboration with the clinical and laboratory endocrine group of Dr. Lynn Loriaux. Dr. D. C. Jimerson, formerly of the Laboratory of Clinical Science, will join the Branch and build a new clinical laboratory for the measurement of classical neurotransmitters and their metabolites, as well as endocrine and peptide substances in plasma and CSF. Receptors for classical neurotransmitters and the new neuropeptides will also be examined in clinically available tissues.

Major new outpatient initiatives have continued within the Section in the past year. Dr. Uhde has established an active and productive clinical research clinic studying and treating patients with anxiety disorders. Dr. Rubinow has begun to study a large population of patients with menstrually-related mood disorders with the aim of not only dissecting underlying neurotransmitter and endocrine alterations, but of using this disturbance as a model for examining rapid and non-pharmacologically-induced alterations in mood and behavior. Dr. F. W. Putnam has broken new ground in his inpatient and outpatient studies of subjects with multiple personality disorder. This previously considered rare psychiatric illness has been described and classified in detail and now appears to be more common than previously thought. Extensive electrophysiological, psychophysiological, and cognitive studies have documented that the subjective

reports of the experience of discrete personality states in these subjects are mirrored by distinct compartmentalization of physiological, psychophysiological, and cognitive processing.

The Section on Psychogenetics continues to have a major focus on outpatient clinical research projects in affectively ill subjects. Novel studies of pharmacogenetics, biological markers in affective illness as well as in the rapidly expanding field of molecular genetics, have been conducted by Dr. E. Gershon. The Section on Clinical Psychophysiology has taken a leading role in Positron Emission Tomography (PET) scan studies utilizing (18F) fluorodeoxyglucose for visualizing and quantitating glucose utilization in brains of normal volunteer subjects and patients with schizophrenic and affective disorders. At the same time, Dr. M. S. Buchsbaum has continued his studies of averaged evoked potential response and EEG electrophysiology, adding new techniques and dimensions of analysis including topographic mapping utilizing 16 EEG channels. This unique interphasing between electrophysiology and functional neuroanatomy using PET scan techniques should prove extremely rewarding in studying psychiatric and neuropsychiatric illnesses.

Unit on Childhood Mental Illness (Dr. Judy Rapoport, Chief)

This Unit conducts research on biological aspects of child psychiatry. Major tools in this research include studies of response to pharmacological agents and use of new neuro-radiological techniques including PET and CAT scans. Because research in child psychopathology is at an early stage of its development, much effort has gone into developing methodology for clinical research. This includes standardization of attentional tasks, of ward settings, and of physiological monitoring such as that for motor activity. Several collaborators from two other Institutes and from NIMH have participated in these studies. The NINCDS (Dr. Denckla) and NIA (Dr. Stanley Rapoport and staff) have been major collaborators. One major focus of study has been the hyperactive child, i.e. those with a diagnosis of Attention Deficit Disorder. A series of studies over the past seven years have addressed the hypothesis of whether a dopamine deficiency exists in this disorder. Recent results have supported the conclusion that noradrenergic mechanisms, rather than dopaminergic, are more likely involved in the hyperactive child syndrome. A variety of pharmacological studies are in progress to further explore the possible neurotransmitter alterations in hyperactive children.

Studies in obsessive-compulsive children indicate that there are CAT scan differences from control groups similar to those reported for some adult schizophrenics. A project with adults, previously diagnosed as having infantile autism, is examining both CAT scans and PET scans in these patients and matched controls. Preliminary results indicate the existence of CAT scan abnormalities, as well as possible increased glucose utilization in deep structures of the brain related to the limbic system.

Recent studies are also examining the effects of dietary substances on behavioral problems in children. Acute and subacute response of normal grade school children to caffeine (10 mg/kg) is being examined. Preliminary results suggest that the degree of habitual caffeine intake in children predicts both baseline differences and response to caffeine. Children who self-select high caffeine diets may be selecting a beneficial, calming agent. Other studies are examining the behavioral response of hyperactive and normal children to glucose

and sucrose. Preliminary findings indicate no disturbance of behavior, even following relatively high dose challenges.

Section on Neuropsychopharmacology (Dr. Daniel P. van Kammen, Chief)

The Section on Neuropsychopharmacology has continued its multidisciplinary approach to the study and treatment of schizophrenia. Studies of high doses of the beta-adrenergic blocker propranolol and the opiate receptor antagonist naltrexone have been conducted to elucidate the possible role of these systems in the schizophrenic syndrome. A role for noradrenergic alterations continues to be supported. Studies of CSF norepinephrine indicate that it is elevated in some paranoid patients. At the same time, platelet alpha-receptor binding is significantly increased. Norepinephrine sensitive prostaglandin E1 stimulation of cyclic-AMP in platelets is decreased, however. Lower CSF dopamine-beta-hydroxylase (DBH) was correlated with altered psychosocial functioning in schizophrenics. Cerebrospinal fluid GABA appears to be significantly lower early in schizophrenic illness, but tends to normalize with increasing duration of illness. Studies with the psychomotor stimulant amphetamine indicate that negative schizophrenic symptoms improve in approximately 75% of the patients studied. These observations are consistent with studies indicating that growth hormone response to apomorphine or amphetamine was decreased in patients with histories of poor premorbid personality. Amphetamine response during pimozide pretreatment predicted early relapse in psychotic symptoms following pimozide withdrawal. CAT scans of schizophrenics show abnormalities in about 20% of subjects studied.

Section on Psychogenetics (Dr. Elliot Gershon, Chief)

The Section on Psychogenetics has as its goals (1) identification of biological and psychological factors that are genetically transmitted in major psychiatric disorders and (2) pharmacologic and pharmacogenetic studies of psychoactive agents in patients with major psychiatric illness and in normal controls.

Cell culture studies have identified a muscarinic cholinergic receptor on the human fibroblast, with similar binding displacment characteristics to the rat brain receptor. There is genetic variation between individuals in the number of binding sites and in the development of increased binding sites after exposure to atropine (up-regulation). Further clinical studies are ongoing.

Pharmacogenetic sleep studies of response to cholinergic agonists revealed heritable differences in REM induction by arecoline in normal twins. This suggests that the previous finding of greater sensitivity to REM induction in affective patients, which is state independent, may be a genetic marker. In awake persons, depressed mood induced by arecoline was not heritable and did not distinguish well state affective patients from normal controls.

A DNA probe has been acquired for proopiomelanocortin (POMC), the precursor protein for ACTH, beta-endorphin, and other peptides of neurobiological interest. Clinical studies of DNA blots from patients are currently searching for polymorphisms. If these are identified, linkage studies will allow us to search the entire POMC gene region for a genetic variant associated with psychiatric disorders.

The theoretical problem of finding optimal strategies for identification of polymorphism and linkage using DNA probes is being explored.

^{3}H-imipramine binding in platelets, previously demonstrated to be genetically variable, has been found not to be different in euthymic, bipolar drug-free patients as compared with normal controls. Other studies found this binding reduced in the depressed state, suggesting it is a state marker and not a genetic trait marker.

Family study and genetic analysis of anorexia nervosa reveal that it is closely related to bipolar and unipolar affective disorder.

Theoretical analysis of recent reports by others of HLA linkage to affective disorder revealed serious methodologic flaws. New data from the Section indicate that this linkage is not replicable.

Section on Clinical Psychophysiology (Dr. Monte S. Buchsbaum, Chief)

During the past year Dr. M. S. Buchsbaum has developed a program using Positron Emission Tomography (PET) with simultaneous topographic electroencephalography. In his first study of regional glucose uptake in unmedicated patients with schizophrenia and age- and sex-matched normal volunteers, he studied individuals resting with their eyes closed. Glucose use was higher in the frontal than posterior lobes in normals, but to a significantly lesser extent in patients with schizophrenia. High frequency, beta activity in the EEG, usually associated with mental activity, was found to be positively correlated with glucose use within normal volunteers.

In a second series of normals and patients, somatosensory stimuli ranging from perceptible to painful were administered during glucose uptake; a third series of normal volunteers viewed flashes of light. The low glucose uptake in the frontal lobes of schizophrenics was confirmed across the total group of 18 patients and 14 controls. Diminished glucose uptake in the region of the basal ganglia and the right parietal lobes was also noted.

With this new metabolic imaging technique has come the need for topographical analyses. Computer programs have been developed by Mr. J. Cappelletti and Dr. Buchsbaum to transform PET slice image files into an approximately equal area projection of the side view of the brain for comparison with similar images produced by EEG or evoked potential topographic mapping.

Anatomic division into lobes or areas allows assessment of glucose or electrophysiology measures on a regional basis. Readout of glucose use under each EEG electrode is also provided. Graphic displays and hard copies of brain maps and superimposition of major sulcal or gyral landmarks allows for publication in scientific journals.

In a parallel program, similar topographic somatosensory evoked potential recordings have been made and effects of psychiatric diagnosis and habituation are being examined.

A new direction for the Section has been initiated by Dr. H. Holcomb in collaboration with Dr. L. Sokoloff's lab. He has assisted in an examination of metabolic changes in the hypothalamus in animal models of dehydration and in the

Dr. L. Skirboll has begun to study the interactions between new peptide transmitter candidates and classic transmitters to examine in greater detail the "cotransmitter hypothesis". These studies are both at the electrophysiological and anatomical levels.

Unit on Studies of Drug Abuse (Dr. David Pickar, Unit Chief)

This Unit, headed by Dr. David Pickar, has recently won recognition as the recipient of the A. E. Bennett Award from the Society of Biological Psychiatry. Drs. D. Pickar, M. Cohen, R. Cohen, and D. Naber received the award for their studies of the effects of the endogenous opioid system in man. During the past year, three projects were completed.

Dr. M. R. Cohen, Guest Worker from the University of Iowa, was principal investigator in a series of experiments in which naloxone in high doses was administered to normal volunteers. The production by naloxone of dose-related increases in self-rated and clinically apparent anxiety and dysphoria support a role for the endogenous opioid system in tonic mood regulation. Similar dose response increases in systolic blood pressure, respiratory rate, and in plasma levels of cortisol and growth hormone also support endogenous opioid system involvement in the tonic regulation of important physiologic and neuroendocrine processes.

The second project was a continuation of the study of radioreceptor assay determined CSF opioid activity in psychiatric patients. This work included collaborations with the research staff of the 2-W, 3-E, 4-E, and 4-W nursing units of the Clinical Center. Recent findings include relationships between CSF opioid activity and self-rated anxiety in normals and observer-rated anxiety in depressed and schizophrenic patients. Cerebrospinal fluid opioid activity was also found to vary with weight in patients with anorexia nervosa, supporting a possible role for endogenous opioids in eating behavior and stress response.

The final area of investigation related surgical stress to plasma levels of beta-endorphin immunoreactivity (ir) in cancer patients undergoing exploratory laparotomies. This work was accomplished in collaboration with Dr. M. Dubois of the NIH Clinical Center Department of Anesthesiology. Plasma beta-endorphin (ir) showed robust increases to surgical stress. Levels of beta-endorphin (ir), stimulated by the surgical procedure, significantly predicted the amount of post-operative morphine administered for pain relief. This latter relationship suggests that changes in plasma levels of beta-endorphin (ir) may relate to endogenous analgesic and stress response systems. The administration of high doses of the potent synthetic opiate, fentanyl, was found to block the beta-endorphin (ir) stress response, suggesting the possibility of important feedback relationships between exogenous opiates and endogenous opioids.

Section on Psychobiology (Dr. Robert M. Post, Chief)

Work in collaboration with Dr. T. W. Uhde has continued to document the clinical efficacy of carbamazepine in the acute treatment of manic and depressed patients. Recent studies suggest that carbamazepine also has important prophylactic effects in patients who did not respond to lithium carbonate. Thus, the work with carbamazepine is of clinical as well as theoretical importance. Systematic investigation of the mechanisms underlying carbamazepine's therapeutic effects in affective illness and their comparison with lithium carbonate may lead

Brattleboro rat. In association with Dr. A. Pert and the Laboratory of Cerebral Metabolism, he has initiated a series of electrophysiological studies designed to demonstrate functional/metabolic connections between various brain nuclei. In particular, they have focused on the substantia nigra and the medial forebrain bundle, both areas related to response to noxious stimulation. Eventually, parallel studies of pain stimulation in rats and man will be performed.

Unit on Sleep Studies (Dr. J. Christian Gillin, Unit Chief)

This Unit which was jointly administered by the Chiefs of the Biological Psychiatry Branch and Adult Psychiatry Branch, has conducted studies of sleep in animals and man. It has made contributions to the basic neuropharmacological control of sleep, nocturnal endocrinology, and clinical use and abuse of sleeping pills. It has studied sleep archectiture and biological mechanisms in affective illness and schizophrenia and also investigated sleep changes in insomnia, childhood enuresis, obsessive-compulsive disorder, Gilles de la Tourette syndrome, the dementias, and adult obsessive-compulsive disorder. A new study of the sleep archectiture of patients with panic anxiety disorders has been initiated in collaboration with Dr. T. W. Uhde. A major investigative thrust of the laboratory has been the documentation in a series of sleep studies that patients with primary affective illness may have a supersensitive cholinergic system. This is evident by the more rapid induction of rapid eye movement sleep episodes following administration of a variety of cholinergic agonists when patients are both in the ill and remitted state. These findings may help to explain some of the sleep changes in affective illness, as well as possibly providing a substrate relating to vulnerability to affective illness. Studies have also been conducted to identify endogenous sleep factors and the interrelationship of sleep and endogenous diazepam receptor ligands. The effects of sleep deprivation and circadian phase alterations have been studied in conjunction with the Section on Psychobiology and the Clinical Psychobiology Branch. Studies in the rhesus primate in collaboration with Drs. C. Kennedy and L. Sokoloff have indicated that glucose utilization is decreased approximately 30% throughout the brain during non-rapid eye movement sleep compared to the awake animal.

Section on Biochemistry and Pharmacology (Dr. John Tallman, Chief)

The Section on Biochemistry and Pharmacology continues its investigations into the mechanisms of action of drugs used in psychiatry.

Dr. J. Tallman has studied the properties of benzodiazepine antagonists and compared their binding properties to those of the benzodiazepines. A method for biochemically determining the properties of unknown compounds has been developed.

Dr. C. Pert has continued her studies of PCP (phencyclidine) receptors and finds that they are identical to one of the subtypes of opiate receptors.

Dr. A. Pert has investigated the effects for morphine on dopaminergic transmission and begun to locally inject PCP into brain regions rich in receptors to study the behavioral properties of these compounds.

Dr. M. Kafka continues her studies into circadian changes in neurotransmitter receptor number and has expanded into the functional aspects of these receptors and their daily alterations.

to new advances in understanding the substrates underlying disorders of affect. Clinical response to carbamazepine has been studied from a pharmacokinetic perspective. Blood levels and CSF levels of carbamazepine itself do not correlate with clinical response. However, carbamazepine-10,11-epoxide, a compound which also possesses anticonvulsant effects, was significantly correlated with degree of clinical and antidepressant response. Clinical and biological markers of response to carbamazepine are being examined, and currently it appears that lithium non-responders and extremely rapidly cycling manic-depressive patients are among those who are benefited by carbamazepine.

The effects of carbamazepine on a variety of classical neurotransmitter and peptide systems are studied. Carbamazepine has complex effects on the dopaminergic system, and appears to exert its antimanic effects through mechanisms other than a direct dopaminergic receptor blockade. In contrast to lithium carbonate, carbamazepine has been used to treat the diabetes insipidus syndrome, and may do so by a direct action at the vasopressin receptor. Carbamazepine significantly decreases CSF somatostatin in affectively ill patients. These data in collaboration with Drs. D. Rubinow and S. Reichlin represent one of the first observations of a psychotropic agent affecting a CSF neuropeptide in man. Carbamazepine did not affect levels of GABA or total opiate binding activity in CSF of our affectively ill patients. While carbamazepine did not significantly affect basal levels of cyclic nucleotides, it tended to decrease the accumulations of these substances following probenecid. The emerging evidence of efficacy of anticonvulsant compounds in the treatment of affective illness raise the interesting paradox of why the major motor seizures of electroconvulsive therapy are also highly effective in this syndrome. Work in collaboration with Dr. F. W. Putnam in the laboratory has indicated that electroconvulsive seizures are paradoxically anticonvulsant to limbic system kindled seizures. These data suggest the possibility that ECS may be clinically effective through mechanisms that also mediate its limbic anticonvulsant effects.

A major focus of work on the Unit has involved assessment of classical neurotransmitter and neuropeptide function in man using CSF methodology. Somatostatin has been documented to be significantly decreased in the CSF of depressed patients in a state-related fashion (Dr. D. R. Rubinow). Somatostatin levels were inversely correlated with those of CSF norepinephrine, a finding of particular interest in relation to the observations by others of the co-existence of norepinephrine and somatostatin in the same neurons. Endocrine markers of affective illness are assessed. Cortisol hypersecretion, as measured by increased secretion of urinary free cortisol, has been shown to correlate with degree of cognitive impairment in depressed patients.

Under the direction of Dr. T. W. Uhde, patients with panic anxiety syndromes are assessed from a clinical and biological perspective and treated in double-blind clinical trials. Clonidine, a specific alpha-2 adrenergic agonist which inhibits firing of the locus coeruleus, significantly decreases anxiety in this population but not in normal volunteers. Alterations in noradrenergic and other neurotransmitter function are being studied in relationship to sleep, physiology, endocrine alterations, pain sensitivity, as well as the subjective experience of anxiety.

Dr. F. W. Putnam has initiated studies of patients with multiple personality syndrome. He has documented changes in EEG and averaged evoked potential response that are more similar within individual personalities than across

personalities in the same subject or in comparison with normal volunteers simulating altered personality states. Psychophysiological studies document that habituation does not generalize across personality states and studies of learning and memory indicate compartmentalization of information processing across personalities consistent with patients' subjective sense of amnesia and lack of information transfer across personality states. These studies may provide new insights into state-related changes in physiological organization of the nervous system of import to behavioral change and the development of psychopathology.

Laboratory studies are examining mechanisms underlying the development of progressive electrophysiological and behavioral alterations to the same stimulus over time. Cocaine-induced behavioral sensitization is environmental context related and affected by conditioning. Brattleboro homozygote rats showed deficient onset, maintenance, and persistence of cocaine-induced behavioral sensitization, while replacement with vasopressin normalizes this defect. In another sensitization paradigm related to kindling, animals demonstrating lidocaine seizures, but not seizures following penthylenetetrazol or ECS, show marked irritable and aggressive behavior, suggesting that this may be a useful model for assessing the relationships of limbic system seizures to altered behavioral pathology.

Dr. G. LaVonne Brown has worked in the Office of the Chief and performed studies in two separate research areas. He has documented a trivariant relationship between a history of aggression/impulsivity, history of suicidal behavior, and low levels of the serotonin metabolite 5HIAA in CSF. In addition to the findings of low levels of 5HIAA with histories of aggression and suicidal behavior, he has also observed similar relationships to low levels of CSF cyclic-AMP and cyclic-GMP. He has also taken a lead in testing the dopaminergic hypothesis of childhood hyperactivity, utilizing dopamine active substances such as piribedil and L-DOPA in double-blind clinical trials in hyperactive children. These data suggest that noradrenergic rather than dopaminergic mechanisms may be important to the therapeutic action of d-amphetamine and related compounds in hyperactive children.

Annual Report of the Clinical Neuropharmacology Branch
National institute of Mental Health
October 1, 1981 to September 30, 1982

Dennis L. Murphy, M.D., Chief

The Clinical Neuropharmacology Branch remains predominantly a small group of clinician-investigators whose major research focus is central neurotransmitter function and the mode of action of psychotherapeutic agents in patients with psychiatric disorders. In addition to their clinical investigations, almost all of our staff spend a significant amount of time involved in studies based on biochemical laboratory approaches or on animal model strategies.

In the last year, as usual, several of our staff were invited to participate in special symposia both in pharmacological areas and in psychiatrically-oriented sessions at major domestic and international meetings. Work accomplished within the Branch led to the recognition of two members of our staff as recipients of the A.E. Bennett award from the Biological Psychiatry Society, Robert M. Cohen in the clinical science division and S. Craig Risch in the basic science division.

Major clinical studies accomplished were in four main areas: (1) A comprehensive investigation of the psychobiology of obsessive-compulsive disorder, and its treatment; (2) a continuation of the studies of patients with affective disorders, principally using substrate-selective monoamine oxidase inhibitors as a way to partially differentiate which of the different monoamine neurotransmitters might contribute to the symptoms and treatment responses in these patients; (3) a pilot study of some features of individuals with psychopathology, particularly affective disorder-related symptoms, associated with the menstrual cycle; and (4) a biological high-risk investigation in normals of whether any consistent psychopathology or other characteristic personality features are associated with abnormalities in smooth pursuit eye movements, a putative biologial marker which prior studies have demonstrated to be associated with schizophrenic and affective disorder patients and their non-ill relatives.

Continuing special emphasis in the Branch has been placed on approaches to the phenomena surrounding long-term adaptations in the central nervous system to chronic stress as a component of most severe psychiatric disorders, and, similarly, on the long-term adaptive processes to psychoactive drug administration. Demonstrated changes in monoamine receptor numbers during antidepressant drug treatment appear to provide a new insight into the mode of action of these drugs, as well as possible models for longer-term changes in neurotransmitter function in response to endogenous factors. In addition to our direct studies of receptor-related functions in animal brain and in non-human primate cerebrospinal fluid amine metabolites, we have concentrated on the pharmacological challenge strategy to assess adaptational processes and central neurotransmitter changes in our human studies. A comprehensive symposium on this subject, which was predominantly the work of the Branch, was published as a series of seven papers in the Journal of Clinical Psychopharmacology in the last year.

Parenthetically, it should be noted that most of the Branch's work (other than some collaborative studies) were carried out in the smallest of the NIMH inpatient clinical wards, and in a two-module laboratory and a one-module non-human primate study area, supplemented by rodent animal care space loaned on a week-to-week basis--as the Branch does not have Building 10 animal space of its own. It is not certain whether the research enterprise was fostered by the fact that six of our professional staff and two research assistants (and often one medical student) all share a single, undivided two-module office. This arrangement at least facilitates frequent cross-communication regarding all of the research projects. It will be of interest to evaluate what the additional space developing from the opening of the ACRF in the next month and the secondary shifts into new space in 18 months or so will have on research production within the Branch.

Brief summaries of the major research accomplishments in the last year are presented below. More detailed discussions and a review of some additional minor projects, especially those accomplished by staff fellows who completed their terms of appointments and have now left the group, are contained in the individual project reports.

Psychobiology and treatment response study of obsessive-compulsive disorder. The comprehensive investigation of individuals with obsessive-compulsive disorder patients directed by Thomas Insel is winding up its first generation of studies and beginning a new series of projects. Thus far approximately 30 patients with this uncommon, treatment-resistant disorder from all over the U.S. have entered the program. Early psychobiological results have suggested a surprising similarity between these patients and patients with affective disorders. Studies of dexamethasone suppression tests, sleep physiology, and receptor sensitivity all reveal biologic abnormalities similar to those seen in depression, even though the obsessive-compulsive patients do not regularly show a disturbance of mood.

Specifically, analysis of the EEG sleep recordings of a 14-patient sample of individuals with RDC and DSM-III-verified diagnoses of obsessive-compulsive disorder revealed significantly decreased total sleep time with more awakenings, less Stage 4 sleep, decreased REM efficiency, and shortened REM latency compared to a group of age- and sex-matched normals. These abnormalities generally resembled those of an age-matched group of depressed patients, although significant differences remained. These findings suggest that such sleep abnormalities as shortened REM latency may not be entirely specific for primary affective illness. They also point to a possible biological link between obsessive-compulsive disorder and affective illness.

In a study of the dexamethasone suppression test (DST) in 16 obsessive-compulsive individuals, 38% were found to have an abnormal response as indicated by an incomplete suppression of plasma cortisol levels. This test has recently received considerable attention as a diagnostic tool for affective illness. While there was a trend for obsessive-compulsive patients with the DST abnormality to have higher depression rating scale scores and a higher family incidence of affective illness than the DST suppressors, none of the obsessive-compulsive patients were diagnosed as having a primary affective disorder. As normals have only a 4% incidence of abnormal responses, and as many other psychiatric disorders such as schizophrenia are not regularly associated with DST abnormalities,

several possible conclusions are suggested: (a) The DST is less specific than earlier studies suggested; (b) patients with secondary depression may have a higher incidence of DST abnormalities than has been indicated by some other studies (which, incidentally, included a few not-well-characterized obsessive-compulsive patients); or (c), as suggested by the EEG sleep studies mentioned above, there may be a closer relationship between primary affective disorder patients and obsessive-compulsive patients than has been previously recognized. To evaluate this and other aspects of familial characteristics of obsessive-compulsive disorder, Carol Hoover is conducting an interview study of the relatives of these patients.

It should be noted that the hypothesis of an association between obsessive-compulsive disorders and the affective disorders is supported by recent reports of the efficacy of tricyclic antidepessants in at least some patients with this disorder, including the preliminary findings from our double-blind, random assignment study indicating that the somewhat serotonin-selective tricyclic, clomipramine, possesses therapeutic effects against obsessive-compulsive symptoms in these patients. It is of special interest that these therapeutic effects do not appear to depend upon depressive symptom reductions in these patients.

A focus for study in the coming year is the possible basis for the response to clomipramine in this disorder. A number of psychophysiologic variables as well as amine metabolites and pharmacologic challenge responses are being studied in obsessive-compulsive patients before and during drug treatment. In addition, the clinical effects of other antidepressants will be evaluated as part of continuing studies of the biology and treatment of this illness.

Studies of the delayed biological and behavioral responses to anti-depressants in affective disorder patients--including evidence regarding central neurotransmitter receptor changes. Among psychoactive agents, antidepressant drugs differ from stimulants like amphetamine or sedatives like barbiturates in the several-week delay in onset of their therapeutic actions. Only minimal clinical changes of any type, except slight sedation, occur during the first 5-10 days of full dose administration, and antidepressant effects may not become evident until 15-25 days after the initiation of treatment. This time lag is a characteristic of antidepressant drugs of widely-divergent chemical structure and of apparently different cellular sites of initial biochemical effects, including the tricyclic and related compounds, the monoamine oxidase (MAO) inhibitors and lithium.

Proposed explanations for this lag time between the initiation of drug treatment and clinical antidepressant response have been many, and range from the time-dependent requirements for the synthesis of new neurotransmitter-related enzymes or other proteins to psychological re-equilibration or relearning processes. Recently, interpretations based upon adaptional changes in neurotransmitter receptor responsivity following longer-term drug administration have been widely discussed. Receptor-based alterations are an especially interesting possibility because prominent receptor changes during antidepressant drug administration have been found in the neurotransmitter systems most clearly implicated in the affective disorders--the noradrenergic and serotonergic systems--and a clear time-dependency upon long-term rather than acute anti-depressant drug administration has been repeatedly demonstrated.

There has been little systematic exploration of time-dependent changes accompanying antidepressant drug treatment in man, other than, of course, the behavioral rating of therapeutic effects and side effects during clinical trials with these agents. In particular, only a handful of studies evaluating the possible involvement of alterations in neurotransmitter receptor function in antidepressant drug responses in man have been reported previously.

Our studies with clorgyline, a selective inhibitor of monoamine oxidase type A (the enzyme form which preferentially affects the metabolism of serotonin and norepinephrine) revealed significant antidepressant effects as measured on both observer-rated and self-rated scales. Although clinical depression ratings had decreased to some extent in the second and third week of clorgyline administration, the changes did not become statistically significant or reach their maximum until the fourth week of treatment. A nearly equal lag period was also observed for another behavioral effect of clorgyline and two other MAO inhibitors, pargyline and phenelzine, the occasional precipitation of excessive behavioral activation in the form of hypomanic or manic episodes--a finding which emerged from a careful record review by David Pickar. All of the patients who developed this side effect did so after a minimum of 22 days of MAO inhibitor administration.

Sleep changes during longer-term MAO-inhibitor administration. The most marked effect of clorgyline on EEG-monitored sleep patterns is a near total suppression of rapid eye movement (REM) sleep. In a recently completed analysis by Robert M. Cohen of sleep recordings made during clorgyline administration, essentially complete (97-100%) REM sleep suppression was found to occur 7 to 10 days following the initiation of drug treatment. Similarly, there was a 7- to 10-day lag period after stopping clorgyline before REM sleep returned to pretreatment levels. It should be noted that although the time course for this change in REM sleep is clearly delayed beyond the 4-24 hour period needed for MAO inhibition to become essentially (>90%) complete, the REM sleep alteration occurs earlier than the mood alterations, raising the possibility of a cascade of changes involving many different mechanisms for these drug effects.

A time lag in the onset of other clinical phenomena besides the mood and sleep changes was also observed with clorgyline. Reductions in systolic, diastolic and mean arterial blood pressure developed in most patients, with a gradual onset during the 4-week period of drug administration. The time course of the blood pressure changes thus tended to parallel the time course of onset of clinical antidepressant effects. In addition, following discontinuation of clorgyline, depressive symptoms began to reappear and blood pressure returned towards pretreatment levels only after a 7-10 day lag time.

Some further evidence for an association between the blood pressure reductions and clinical antidepressant effects was found in the significant positive correlation ($r = 0.58$, $p < 0.05$) between the reduction in Hamilton depression scale ratings and the reduction in standing blood pressure in the final week of clorgyline administration. Correlation coefficients of similar magnitude between blood pressure changes and therapeutic effects have also been found for several of the self-rated scales used in this study, including the Beck Depression Scale and the POMS depression factors.

Changes in the pressor response to clonidine during longer-term antidepressant drug administration. A more direct examination of the question of possible processes involved in the different time-dependent effects of MAO inhibiting antidepressants has recently been conducted by Larry Siever using a pharmacologic challenge approach to assess the status of the noradrenergic neurotransmitter system in man. This particular study utilized clonidine, a centrally-acting adrenergic agent which in low doses produces a hypotensive response. This blood pressure response appears due to clonidine's action as an α_2-adrenergic agonist since hypotension can be induced in animals by direct application of clonidine to the locus ceruleus, where α_2-adrenergic autoreceptors mediate an inhibitory feedback effect on noradrenergic activity.

Depressed patients examined prior to clorgyline administration manifested a reduction in mean arterial pressure following a single clonidine dose. When clonidine administration was repeated after four weeks' treatment with clorgyline, nearly complete blockade of the clonidine hypotensive response occurred. However, after clorgyline administration for only three days, a hypotensive response equal to that found in the control, pre-clorgyline period was observed. Overall, a consistent, highly statistically significant attenuation of the clonidine response has been found during longer-term but not short-term clorgyline administration to nine patients, with an intermediate level of blockade observed after ten days of treatment. These data provide evidence that the antagonism of the clonidine response may well represent a subsensitization of brain α_2-adrenergic receptors resulting from chronic but not acute monoamine oxidase inhibition.

Parallel studies in animals were conducted by Robert M. Cohen specifically examining changes in brain noradrenergic receptors during the acute, semiacute and chronic administration of clorgyline and other MAO inhibitors. Significant reductions in numbers of β-adrenergic receptors (assessed using ^{3}H-dihydroalprenolol as the binding ligand) were found following 21 days of treatment with clorgyline and also with the non-selective MAO-inhibitor phenelzine, but not with the MAO-B inhibitor pargyline (which, although structurally similar to clorgyline, acts only as a weak inhibitor of norepinephrine degradation and has only weak antidepressant effects). β-Adrenergic receptor numbers were unaltered after three days of treatment with clorgyline, while after 10 and 21 days, maximum reductions were observed. Decreased numbers of α_1-adrenergic receptors measured with ^{3}H-WB4101 were also observed following a minimum of ten days' treatment. Reductions in α_2-adrenergic receptors estimated from ^{3}H-clonidine binding were 14% at 3 days, 35% at 10 days and reached a maximum reduction of 62% at 21 days--a quite remarkable parallel finding to the evidence from the series of clonidine administration studies carried out in patients.

The sequence and interrelationships among these receptor changes has only begun to be evaluated. The evidence for reductions in adrenergic receptor numbers observed during antidepressant drug administration supports the clinical evidence of a reduction in central sympathetic outflow as reflected in decreased plasma norepinephrine concentration and decreased blood pressure, apparently in compensation for the acute effects of the drugs. Such an interpretation would be in keeping with the revised hypotheses for depression invoking dysregulation of catecholamine functions and, in one version, postulating an overactive, inefficient state of catecholaminergic synaptic function in endogenously depressed patients. Alternatively, the functional state of the central noradrenergic

system might be interpreted as being re-equilibrated by the changes produced by antidepressant treatment, perhaps establishing a new more beneficial steady state. While our evidence is quite preliminary, these studies illustrate the potential for illuminating possible mechanisms of action of antidepressant drugs in man, and further suggest the crucial importance of examining and correlating the sequential development of biological and behavioral changes during the course of chronic treatment with other drugs in patients.

Brain noradrenergic receptor status in depression. As an approach to the assessment of the functional state of the central noradrenergic neurotransmitter system in depressed patients, the physiologic response to intravenously-administered clonidine has been used in a series of pharmacological challenge studies in psychiatric patients and controls. Clonidine selectively affects brain α_2-adrenoreceptors, having no serotonergic or dopaminergic effects. While these α_2-adrenoreceptors are present both post-synaptically and pre-synaptically, clonidine's major pharmacologic effects (and its clinical use as an antihypertensive drug) are thought to depend on its presynaptic, i.e., auto-receptor effects, which lead to a reduction in firing rates in noradrenergic neurons in the locus ceruleus, and a consequent reduction in sympathetic outflow associated with reductions in blood pressure, pulse, and plasma norepinephrine concentrations, as well as reductions in catecholamine metabolites such as MHPG. Clonidine also affects neuroendocrine function, acting to raise plasma growth hormone concentrations in man. The mechanism of this latter effect is not clear, although growth hormone release in man is known to be altered by other noradrenergic agents; the α-adrenergic receptor blocker, phentolamine, for example, reduces growth hormone levels produced by a number of stimuli.

In a recently completed study comparing growth hormone responses to clonidine in affective disorder patients and controls conducted by Larry Siever, the depressed patients as a group showed a significant blunting in their responses to clonidine. This difference remained equally significant when only patients who were age- and sex-matched with controls were used in the comparison. This finding substantiates the two earlier smaller studies reported by Matussek and coworkers and Checkley and coworkers, and a third study published simultaneously with ours by Charney and coworkers, which also described reduced growth hormone responses to clonidine in depressed patient groups. This finding is thought to reflect a noradrenergic response difference rather than a difference in other mechanisms affecting growth hormone release, since normal growth hormone responses to the dopaminergic agonist, apormorphine, have been observed in other studies of depressed patients. In addition, there is evidence of reduced growth hormone responses to agents such as amphetamine, insulin-induced hypoglycemia and desipramine, which also may act through adrenergic mechanisms, although less specifically so than clonidine.

Associated studies indicate that the magnitude of the growth hormone response to clonidine corresponds inversely to pretreatment plasma MHPG concentrations in the depressed patients, suggesting that increased noradrenergic release may be associated with decreased α_2-adrenergic responsiveness, a finding predicted from basic principles of receptor adaptation. We also have preliminary evidence that clonidine may induce a decrement in plasma cortisol concentrations, and that this decrement may be greater in depressed patients than controls. We are pursuing this lead to further explore the relation of plasma cortisol to the noradrenergic system.

Affective symptoms associated with the menstrual cycle. Jean Hamilton initiated a series of investigations of menstrual-cycle related affective disorder during the last year. Approximately 11 subjects (5 with affective disorder) with self-identified severe premenstrual affective symptoms have entered the program through the outpatient clinic or the inpatient unit, while another group of 8 symptomatic subjects were identified from an initial sample of 97 women volunteers who were screened for the presence of menstrual cycle-related symptoms apart from other evidence of psychopathology. Another 16 non-symptomatic volunteers and screened subjects form a third comparison group.

Subjects in these index and control groups have been evaluated on a number of mood, behavioral, biological and clinical measures in the premenstrual versus intermenstrual phases of the cycle. Although only preliminary data are available, it appears that the self-identified population may differ from the screened group when compared on daily mood and personality characteristics. In other investigations in these subjects, neuroendocrine responsivity to clonidine versus placebo challenges in pre- and post-menstrual phases of the cycle are being compared across subject groups as possible aids in identifying subgroups. In view of a recent hypothesis linking β-endorphin changes to premenstrual symptoms and the finding that clonidine suppresses opiate withdrawal symptoms, these studies may have implications for developing approaches to treatment. Since we are using the night-time melatonin response to clonidine as an endpoint measure, another project has included the evaluation of the natural pattern of urinary 6-hydroxy melatonin excretion during the menstrual cycle in six normal subjects, in collaboration with Sanford Markey.

Further studies in affective disorder populations. Other projects dealing with the assessment and treatment of patients with affective disorders included the completion of a major review of rating scale methods for the evaluation of depressive and manic symptoms and the completion of a series of reviews of the pharmacological challenge approach to evaluating the functional status of the different central neurotransmitter and neuromodulator systems in man. Several reviews of various aspects of the clinical uses of monoamine oxidase inhibiting antidepressants were also completed, along with a review of the question of possible relationships between antidepressant drug responsiveness and diagnostic subgroups among affective disorder patient populations.

A biological high risk strategy study of abnormal smooth pursuit eye movements in relation to psychopathology. As in several previous and ongoing studies conducted in this Branch, a high-risk study approach to the evaluation of possible correlates of psychopathology is currently being carried out by Larry Siever using abnormal pursuit eye movements as the biological variable. Poor visual tracking of a swinging pendulum target has been shown repeatedly to be a characteristic which distinguishes schizophrenic patients and their well relatives and affective disorder patients from controls.

Two hundred eighty volunteers have been screened, and the accuracy of their smooth-pursuit eye movements assessed. Subgroups of individuals with low accuracy tracking and high accuracy tracking have been identified and have undergone psychiatric interviews including the Schedule for Affective Disorder and Schizophrenia (SADS) and extensive psychologic testing.

Preliminary data indicate that low accuracy trackers show evidence of more psychopathology than high accuracy trackers according to psychiatric interview data obtained by staff blind to their eye-tracking status. Nine of 11 individuals satisfying DSM-III criteria for Schizotypal Personality Disorder were in the low accuracy tracking group, as were all eight individuals with a history of hypomania established by Research Diagnostic Criteria (RDC). Further correlations with clinical ratings indicated significant associations between the degree of accuracy of tracking and characteristics of flattened affect, decreased rapport, referential ideas, suspiciousness, brief delusions, social isolation, dependency, and preference for being alone.

Results from this study are still being analyzed, but suggest overall that low accuracy tracking is associated with schizophrenia-related and mania-related traits. In these subjects with schizophrenia-related traits, the results reflected the profile of an individual with subtle neurologic dysfunction, poor perceptual boundary differentiation, decreased affect and social isolation with avoidance of interpersonal intimacy. We have now retested the majority of low accuracy trackers and find that 11 out of the 13 subjects who show low accuracy tracking under laboratory conditions manifest schizotypal characteristics.

We also have studied the correlation between inaccuracy of tracking and the behavioral response to amphetamine in psychiatric patients, to test the hypothesis that arousal may differentially alter physiological functions in these individuals. These results suggest that low accuracy trackers improve their tracking with amphetamine while it deteriorates in individuals with high accuracy tracking. Correlations between inaccuracy of tracking and schizotypal-related characteristics and other biologic variables in various psychiatric patient groups including obsessive-compulsive individuals, patients with affective disorders and schizophrenia are being evaluated in collaboration with other NIMH groups.

Laboratory physicochemical studies of mechanisms for monoamine storage and metabolism. The work of Jonathan Costa continues to explore factors regulating amine uptake, metabolism, storage, and release in platelets and other amine-storing tissues, as well as various types of model systems. The requirements for calcium transport, important in the formation of amine storage granules and the exocytotic release of amines, have been examined in model systems and intact mitochondria. Mitochondria have also been shown to be affected by certain psychoactive drugs, which can alter respiration, ATPase activity, and calcium transport. The enzyme phenolsulfotransferase appears to play a role in amine metabolism in platelets, nerves, and microvessels (capillaries). Amine uptake in platelets is sensitive to the extracellular glucose level and a number of steric and electronic constraints on the indole ring. The mechanisms responsible for vesicular amine storage in human platelets do not appear to involve amine binding or an amine re-uptake system, although selective vesicular membrane permeability appears to play an important role. The amine storage complex in chromaffin vessels, dopaminergic nerves, and platelets from humans and from other species, as evaluated in intact tissue by nuclear magnetic resonance, has been found to have unique motional properties. Many of the studies in platelet and nerve ending particles have been facilitated by the availability of a variety of ring-fluorinated amines, and as a consequence a good deal of new information has been obtained about the biochemical and

biophysical behavior of these fluorinated compounds. Since other work has shown that these substances appear relatively resistant to electron irradiation, it should be possible to accurately define their subcellular distribution. Photons as well as electrons may be useful for this purpose, either through contact X-ray microscopy, scanning X-ray microscopy, or X-ray laser holography.

Annual Report of the Clinical Psychobiology Branch
National Institute of Mental Health
Thomas A. Wehr, M.D.

This has been a year of transition for the Branch. Dr. Frederick K. Goodwin, Chief of the Branch, was appointed Scientific Director of the Intramural Research Program of the Institute. Dr. Thomas A. Wehr, Chief of the 4-West Clinical Research Unit, is Acting Chief of the Branch. Several investigators are leaving the Branch to take on new responsibilities elsewhere. Dr. Steven Paul, who for several years was Chief of the Unit on Preclinical Pharmacology, will become Chief of a new branch, the Clinical Neurosciences Branch. Dr. Paul's laboratory projects (ZO1 MH 01836-04 CP) will be transferred to that Branch. Dr. Rex W. Cowdry and his projects will join Dr. Paul's Branch. Dr. Paul J. Marangos, Chief of the Unit on Neurochemistry, and his program (ZO1 MH 01834-05 CP, ZO1 MH 01833-02 CP, and ZO1 MH 01831-06 CP) will be transferred to the Section on Histopharmacology under Dr. David Jacobowitz in the Laboratory of Clinical Science. Dr. William Z. Potter, Chief of the Unit on Clinical Psychopharmacology, will leave the Branch to become Chief of a Section on Clinical Pharmacology under the Director. Dr. Markku Linnoila will continue to work with Dr. Potter. Dr. Philip W. Gold will leave the Branch to become Chief of a Section on Neuroendocrinology under Dr. Robert M. Post, Acting Chief of the Biological Psychiatry Branch. Finally, Dr. Alfred J. Lewy has left the NIH to become Assistant Professor at the University of Oregon. Dr. Lewy will continue to collaborate with members of the Branch.

The Clinical Psychobiology Branch, under its Acting Chief, Dr. Thomas A. Wehr, will continue to direct its primary research effort towards understanding the spectrum of affective disorders and their treatment. A major theme of the Branch as it is presently organized will be the role of biological rhythms and sleep in the pathophysiology of affective disorders. To promote the integration of the related fields of circadian rhythm and sleep physiology, the Unit on Sleep Studies, under its new Chief, Dr. Wallace Mendelson, will be merged with the Branch. This arrangement, which involves physical relocation of the sleep lab to the 4-West Clinical Research Unit, will provide better conditions for sleep monitoring and will bring together the resources necessary to support more rigorous and sophisticated studies of sleep and circadian rhythms in special environmental conditions where subjects can be isolated from external 24-hour time cues.

The Branch will continue to pursue basic laboratory research related to the major themes of its clinical research. Dr. Gerard Groos has established a neurophysiology laboratory in which neural activity of central circadian rhythm pacemakers can be studied directly. Dr. Mendelson will begin a series of experiments designed to elucidate mechanisms and pathways by which circadian pacemakers control the sleep-wake cycle. The neuropharmacology of the circadian system (especially the effects of antidepressant drugs on circadian pacemakers) will continue to be a focus of basic research.

PROJECT SUMMARIES

The following summaries reflect the major research projects of the Branch during the past year. They are described in greater detail in the accompanying project reports.

The Clinical Research Unit is a thirteen bed inpatient unit devoted to the study of major affective disorders. This unit is the primary focus for the clinical research of the Branch; it provides comprehensive psychiatric care for patients involved in our research projects. Under Dr. Wehr's direction the Unit screens and admits patients, gathers medical, psychiatric, and psychosocial information, and coordinates the efforts of the Branch's researchers to provide an appropriate evaluation and treatment program geared to the clinical needs of the patient and the research interests of the Unit.

Traditionally, the emphasis of our specialized resources has been the longitudinal study of episodes of affective illness, before, during, and following a variety of therapeutic interventions; we have had a special interest in patients with rapidly cycling bipolar illness. The "brief inpatient evaluation" program for depression, in which outpatients are admitted to the Unit for one week of pretreatment studies and are then discharged to be treated as outpatients, is now entering its third year. This approach enables us to evaluate a much broader spectrum of depressive disorders using the methodologies previously applied to the endogenous depressions without seriously compromising our ongoing longitudinal studies of cycling bipolar illness. This program has been highly successful in providing an increased number of patients for a variety of projects. Particularly noteworthy in this regard is the large number of patients with recurrent winter depressions who were studied and treated this past year. All patients were admitted to the unit for a battery of research procedures in the summer and in the winter. The efficiency and competence of the nursing and medical staff made it possible to conduct a large number of individual studies in a relatively short time, and to maintain uniform environmental conditions so that results of summer and winter studies could be compared.

The rapid increase in the number of patients and in the variety of projects has changed the clinical character of the Unit and its milieu. In general the medical and nursing staffs have responded well to these changes. Clearly more clinical research is being successfully accomplished than ever before. Despite increased diversity of projects, the continuous collection of standard data provides a solid basis for comparability. Thus, behavior and mood are regularly rated by patients and nursing staff, blood and urine collections are scheduled on a regular basis, motor behavior is continuously monitored by solid-state activity monitors, and sleep is recorded nightly. Within this general framework specific evaluation and treatment protocols are introduced. Evaluation protocols include detailed studies of circadian rhythms, neuroendocrine challenge tests, sleep recordings using the electroencephalogram (EEG), and cerebrospinal fluid collections. Some treatment approaches are non-pharmacologic, such as sleep shifts, sleep deprivation, high intensity light exposure, and ECT. Pharmacologic studies have focused on low dose clorgyline (a specific inhibitor of MAO-A) for treatment of lithium-resistant, severe, rapidly cycling bipolar illness.

Finally, to provide a control population for our research in affective

illness, the Unit admits and studies "normal volunteers," using many of the research paradigms applied to affectively ill patients.

Biological Rhythms in Affective Illness continues as a major focus of the Clinical Research Unit under the direction of Dr. Thomas A. Wehr. There is now considerable evidence that timekeeping by a biological clock that controls circadian rhythms is abnormal in affective illness and that this abnormality may play an important causal role in the illness. Circadian rhythms are near 24-hour patterns of variation in behavior and physiology. They are generated by a clock-like pacemaker in the hypothalamus and are synchronized with the external day-night cycle by environmental stimuli that have the properties of time cues. Our previous studies have indicated that the timing of circadian rhythms is shifted several hours earlier than normal in depressed patients. We also found that when manic-depressive patients switch from the depressed to the manic phase of their illness most experienced from one to ten abnormal double length 48-hour sleep-wake cycles. Double length sleep-wake cycles also occur in normal subjects who are experimentally isolated from external time cues, and may result from an abnormal slowing of the intrinsic rhythm of the pacemaker that drives the sleep-wake cycle, such that it escapes from its normal 1:1 mode of coupling to the day-night cycle to a 1:2 mode. Studies during the past year have shifted increasingly from descriptive studies to experimental studies of the circadian system. These experimental studies are designed to test hypotheses about the possible role the circadian system plays in the cause of manic and depressive episodes.

Major findings this year include the following:

Compared with normal controls, depressed patients' rectal temperature minimum often occurs abnormally early during their sleep period. This abnormality may indicate that the timing or phase position of a circadian rhythm or pacemaker is shifted abnormally early in depression, and could partly explain other abnormalities such as the characteristic early temporal distribution of REM sleep and early awakening of the depressive.

Theoretically the abnormal phase-relationship between circadian rhythms and sleep in depression could be corrected by advancing the timing of the sleep period several hours earlier than normal. In this case the patient would be sleeping at an abnormally early time relative to external clocks but at a more nearly normal time relative to their internal clock. We showed previously that depressed patients could be temporarily brought out of their depressions by advancing the time of their sleep period from 11pm-7am to 5pm-1am. This year three additional patients participated in similar experiments. Two patients switched out of depression after 2 days on the experimental protocol and remained undepressed for several months. These results, together with those of other investigators who have studied the effects of partial sleep deprivation on depression, suggest that (1) depression depends on a process that occurs during sleep, and (2) depression depends on sleep occurring at a critical early morning circadian phase. In the coming year these related hypotheses will be explored in an experiment in which patients' sleep will be restricted to 2 different parts of the night that are predicted either to cause or to relieve depressive symptoms. In addition to providing a test of our hypotheses, these experimental sleep regimes may prove to be effective and practical long-term antidepressant

treatment.

In animals the circadian system is centrally involved in the regulation of seasonal behavioral rhythms. Animals detect changes in day length and use this information to snychronize their annual rhythms with the solar year. Day length is measured via the retinohypothalamic tract which detects light and the suprachiasmatic nucleus (the circadian clock), which measures time.

This year we identified several thousand patients who experience recurrent winter depressions. We hypothesized that these depressions were triggered by the same environmental factor that triggers seasonal changes in behavior in animals--changing day length. In order to test this hypothesis, we experimentally treated 12 patients by extending their winter days with bright artificial light (in a randomly ordered crossover comparison with dim artificial light). Nearly every patient responded to the active light treatment within 2-3 days, and relapsed when lights were withdrawn. These results provide preliminary evidence that such seasonally recurring depressions depend on interaction of annual changes in environmental light with a time-measuring photosensitive mechanism in the circadian system. This hypothesis will be subjected to a more rigorous test with a larger number of patients during the coming year. Results that support the hypothesis would link this particular manifestation of depressive illness to a rich body of animal research concerning the regulation of behavioral cycles by light.

We completed a large scale longitudinal study of locomotor activity (the activity-rest cycle) in rapidly cycling manic-depressives. Using a small self-contained electronic wrist activity monitor developed at the NIMH, we found that such patients experience one-to-ten 48-hour sleep-wake cycles when they switch from depression to mania. In order to test whether these abnormal double length sleep-wake cycles help to cause patients to switch out of depression we asked 12 depressed rapidly cycling patients to simulate a 48-hour sleep-wake cycle by remaining awake for 40 hours (one night's total sleep deprivation). Most of the patients switched out of depression into mania or hypomania, which sometimes lasted days or weeks. These results support the hypothesis that the abnormal 48-hour sleep-wake cycles help to trigger spontaneous switches out of depression into mania. Subsequent experiments showed that the sleep deprivation response (1) could be blocked by lithium, a drug that prevents mania, and (2) did not depend on the presence of light during the sleep deprivation night.

Continuing animal experiments have confirmed that antidepressant drugs have powerful effects on biological clocks and that these effects are in the direction predicted by hypotheses about circadian rhythm abnormalities. Dr. Groos has extended to a larger group of animals his original finding that the drugs appear to act directly on the suprachiasmatic nucleus (SCN), the small group of cells that function as a central circadian pacemaker. Firing rates of the same light-responsive cells in the SCN were found to be inhibited by both antidepressant drugs and serotonin. Future experiments will investigate the possibility that effects of antidepressant drugs on the rhythm of the SCN depend on serotonergic mechanisms.

The most ambitious and fruitful studies of the human circadian system have

employed experimental conditions where circadian rhythms could be monitored in isolation from external influences arising from schedules and environmental time cues. In order to measure the intrinsic behavior of circadian pacemakers free of external influences in patients and normal volunteers, we have set up 2 temporal isolation facilities on our research unit. One manic-depressive patient has been studied in temporal isolation for 3 weeks. There were two interesting findings: (1) although she had been depressed for six months, she switched out of depression after 3 days in temporal isolation, possibly because the timing of her sleep relative to her circadian rhythms radically changed in these conditions; (2) a circadian pacemaker ran abnormally fast (faster than 1 cycle/24 hours), as had been predicted by one interpretation of the phase-advance hypothesis of depression.

The biological rhythm studies have led to new hypotheses about the causes of mania and depression and about the mechanisms of action of drugs used to treat these disorders; concepts drawn from basic biological rhythm research have also led to two novel non-pharmacological treatments of depression.

Melatonin studies in humans have played an important role in our studies of biological rhythms and effects of light in patients with affective disorders following the earlier development by Drs. Lewy and Markey of a highly sensitive and specific gas chromatographic-mass spectrometric (GC-MS) assay. This assay permits us to conduct clinical research on the circadian pattern of melatonin secreted by the pineal gland and on the inhibitory effect of different intensities of light on melatonin secretion. Because Dr. Lewy has left the NIH, the melatonin project per se will be terminated, although members of the Branch will continue to collaborate with Dr. Lewy, who has set up both GC-MS and RIA assays for melatonin at the University of Oregon.

Major findings this year include the following:

We have replicated our earlier findings that manic-depressives' nocturnal melatonin secretion is abnormally sensitive to inhibition by light. We had previously found that very bright artificial light, but not light of ordinary intensity, can suppress nocturnal secretion of melatonin in normal subjects. In contrast to normal subjects, manic-depressives appear to be very sensitive to low intensity artificial light (500 lux). Our replication studies confirmed that their hypersensitivity to light is independent of clinical state and season of the year. This abnormal sensitivity to light of the retinohypothalamic tract-suprachiasmatic nucleus-pineal complex could be partly responsible for circadian rhythm disturbances seen in both mania and depression (timing of circadian rhythms is partly regulated by environmental light).

The importance of light in the phase control of circadian rhythms in humans is highlighted by the results of our studies of circadian rhythms in melatonin secretion and body temperature in blind persons. We previously found that blind persons' melatonin rhythms were synchronized with the day-night cycle with markedly abnormal phase relationships (e.g. secretion was maximal in the daytime rather than at night) or were actually free-running, that is, no longer entrained to the 24-hour day-night cycle, but following their own approximately 25-hour rhythm and gradually going in

and out of phase with the solar day. These findings were extended to eight more blind patients during the past year.

As part of the biological rhythm project, melatonin will continue to be used as a marker of the oscillations of a central circadian pacemaker and of that pacemaker's sensitivity to light.

Amine neurotransmitters and metabolites. The purpose of this project is the direct assessment of the functional state of brain neurotransmitter amines in patients and normal persons by assay of levels of the amines and their major metabolites in plasma, in urine, and in the cerebrospinal fluid (CSF). The project has been greatly aided by the development of new HPLC methods for the measurement of transmitters and metabolites. These methods offer the advantage over GC-MS methods that we can measure both neurotransmitters and their metabolites in a single sample using a single methodology. The availability of essentially simultaneous measurements of monoamine neurotransmitters and their metabolites makes it possible for the first time to study complex interrelationships between neurotransmitter release, reuptake and degradation in single neurotransmitter systems, and the relationships between catecholamine and indoleamine neurotransmitter systems.

Significant findings this year include:

The principal new clinical findings are extensions of previous studies associating serotonin metabolism with impulsive or violent behavior. A retrospective chart review of patients with major depressive disorder studied on our inpatient unit over the past eight years suggests that there is a significant correlation between low levels of CSF 5-HIAA following probenecid administration and the occurrence of significant episodes of behavioral dyscontrol at some point during the patient's lifetime. Also, Dr. Linnoila, in collaboration with colleagues in Finland, found that psychopathic murderers have low concentrations of 5-HIAA in CSF, in contrast to individuals who commit murder while experiencing paranoid thoughts, who have normal levels of 5-HIAA than those who are not suicidal. These findings suggest that altered serotonergic function may correlate with behavioral dyscontrol, regardless of diagnostic category.

HPLC studies of monoamine metabolites in urine showed that high phenylethylamine (PEA) excretion rates are associated with delusional states in rapid cycling bipolar patients. Perhaps most significant is the finding that five different antidepressant treatments, including lithium and electroconvulsive therapy, all reduce total norepinephrine metabolism in depressed patients, suggesting a common biological effect for apparently dissimilar treatment modalities. Thus the postulated importance of norepinephrine systems in the pathophysiology and treatment of affective disorders continues to receive indirect support.

Dr. William Potter coordinates the Branch's research involving the Clinical Pharmacology of Antidepressants. HPLC assay development has continued to expand dramatically with the addition of Dr. Markku Linnoila to our staff and with the participation of two Visiting Fellows, Dr. Scheinin from Finland and Dr. Zhang, Wen-ho from the People's Republic of China. New developments pertinent to the studies of amine neurotransmitters and metabolites in mental illness are

discussed above. Modifications of the same system are used to measure neurotransmitter changes in brain, CSF, and blood from animal models. We are finding that the validity of rodent models to assess drug-induced biochemical effects has major limitations (see below).

Pharmacokinetic studies provide a means of studying crucial variables in drug response by assessing absorption, distribution, and metabolism of the drug, and by relating steady-state levels of the drug to observed biological and behavioral effects. Extension of previously initiated studies as well as new findings include the following:

Single dose administration of DMI to predict the dosage required to achieve satisfactory "steady-state" plasma levels continues to be used successfully in studies involving normal volunteers and elderly women. Recent studies have shown that across a wide age range (from 20 to 90 years) the renal clearance of active hydroxy metabolites of tricyclic antidepressants decreases in parallel with the known decrease in GFR with age. At the same time, the finding that in otherwise healthy individuals, metabolism of tricyclics does not decrease with aging has been replicated and extended.

A cross-racial Chinese/Caucasian comparison of pharmacokinetics following single oral dose desipramine reveals a several-fold greater incidence of "slow hydroxylators" among the Chinese. This finding could explain anecdotal reports that Chinese require lower doses of both tricyclic antidepressants and drugs such as propranolol, whose elimination is dependent on hydroxylation.

Biochemical investigations of the effects of antidepressants on the noradrenergic system in man have been successfully extended so that five distinct organic treatments have been assessed in depressed patients:

ECT, lithium, clorgyline (a selective MAO Type A inhibitor), desipramine (a selective norepinephrine uptake inhibitor) and zimelidine (a selective serotonin uptake inhibitor) have all been found to reduce either norepinephrine, its major metabolites or both consistent with an interpretation that all antidepressant treatments affect the noradrenergic system in a manner indicative of increased effective intrasynaptic norepinephrine. A corollary would be that marked effects on the noradrenergic system may be necessary but not sufficient for antidepressant effect since many patients did not improve clinically despite marked biochemical change.

An interesting related finding indicating the importance of other variables is that desipramine produces similar effects on norepinephrine in subjects of all ages (9-90 years) but only produces cardiovascular changes in subjects under 65 years. Whether this decreased peripheral physiologic response is paralleled by decreased CNS responsivity in the elderly to identical biochemical stimuli is currently being explored.

Reassessment of interpretations of the biochemical consequences of MAO Type A inhibition based on substrate specificity in rodents is needed in light of results following the administration of clorgyline. Low doses produced marked reductions of the deaminated metabolite of norepinephrine in urine as

expected but not of the serotonin metabolite. Preliminary evidence in CSF from monkeys given low dose clorgyline also show a specific effect on norepinephrine metabolism showing that in primates MAO substrate specificity is different from rodents in which serotonin metabolism is markedly reduced following clorgyline.

Clinical studies have proceeded in three main areas:

The antidepressant and anticycling effects of clorgyline have been confirmed. In low doses this selective MAO Type A inhibitor provides a potent therapeutic agent with impressive advantages over available mixed-action MAOIs. Consistent with the interpretation that this drug is acting through effects on norepinephrine, it has been found to induce or aggravate hypomania in all bipolar subjects thereby requiring combination with lithium for maximum benefit.

We have observed that some depressed patients with atypical characteristics alos had several-fold elevations of phenylethylamine (PEA) in urine and subsequently prospectively identified subjects with a similar clinical presentation and elevated PEA. The possibility that there is a high PEA subtype of depressive disorder is under investigation. Initial experience with a controlled trial of carbidopa, a peripheral decarboxylase inhibitor which reduces PEA formation, is promising in that a relatively specific treatment may be available for these patients.

Single doses of amitriptyline, desipramine or zimelidine alone or in combination with alcohol have been administered to volunteers to assess performance on a sophisticated psychomotor battery of tests. Whereas amitriptyline was clearly additive to alcohol in terms of deleterious effects and desipramine relatively free of interaction, zimelidine not only counteracted alcohol's effects on information processing but by itself improved performance in this area. This finding is particularly exciting in light of a recent clinical report that the serotonin uptake inhibitor zimelidine is particularly efficacious in terms of impairing cognition in depressed patients. Chronic trials have been initiated to test this effect of zimelidine.

Neuroendocrine studies of major psychiatric disorders have continued under the direction of Dr. Philip W. Gold. The rationale for these studies is as follows: First, several aspects of the symptom complex of the functional psychoses, particularly affective illness, suggest hypothalamic dysfunction. Second, since monoamine neurotransmitters modulate the synthesis and release of hypothalamic peptides and pituitary hormones, endocrine changes in plasma can be used as a measure of the functional activity of biogenic amine systems. Third, peptides and hormones have specific receptors in brain, affect brain neurotransmitter systems, and elicit specific behavioral responses. Dr. Gold has established an extensive collaborative relationship with the Developmental Endocrinology Branch of the NICHD. Clinically this collaboration focuses on pathophysiological analogies between depression and Cushing's syndrome. In the laboratory Dr. Gold has participated in the development of radioimmunoassays for a variety of peptide hormones.

Major findings this past year are as follows:

Our previous finding that CSF arginine vasopressin (AVP) is lower in bipolar depressed patients compared with manic patients and normal controls, has been replicated in a larger number of patients. In contrast to bipolar patients, unipolar patients' AVP function appears to be normal. Measures of peripheral and central AVP dysfunction were highly correlated in the bipolar patients. Several psychotropic drugs used to treat bipolar patients were found to alter central AVP function in a manner opposite to their effects on peripheral AVP function. Abnormalities in AVP function have also been found in schizophrenic and anorexic patients. DDAVP, a synthetic AVP analogue, was found to enhance cognition and memory in patients with Alzheimer's disease.

A variety of static and dynamic tests have documented abnormalities in oxytocin, somatostatin, gonadotropin and prolactin responses in affective and schizophrenic patients.

A major new project involves investigation of the common neuroendocrine and neurobiological aspects of depression and Cushing's disease. An important focus is on potential clinical applications of the newly sequenced corticotropin releasing hormone to the diagnosis and treatment of adrenal and affective disorders. Studies in primates indicate that CRH elicits several physiological and neuroendocrine responses that are characteristic of the stress reaction.

Outpatient follow-up studies of manic-depressive patients and families have been conducted for the past eight years by Mrs. Yolande Davenport and others in the Unit on Family Studies and the Outpatient Unit. Studies focused on patients participating in long-term lithium maintenance psychotherapy groups have been closed out. Current projects involve assessment of family dynamics in specific newly recruited subgroups of affective patients, rapid cyclers and seasonal depressives. Another project involves investigation of development and nurturing styles in parent-child relationships where the parent is affectively ill.

Significant observations this year include:

Defensive techniques were compared in families of rapidly cycling and non-rapidly cycling manic-depressives. The principal findings were that families of rapid cyclers experience more pervasive and chronic anxiety because of unremitting fear of recurrence of the illness, and there is more role diffusion among family members perhaps because of ever-changing capacity of the patient to function and the changing requirements and responsibilities this places on other family members. This phenomenon, which we have termed "family cycling" illustrates very dramatically the family morbidity of affective illness.

A follow-up study of rapidly cycling manic-depressives is under way. Previous reports at another center indicated that this type of patient had an especially poor prognosis. Preliminary findings in our study indicate that many patients with a history of rapid cycling have done surprisingly well, apparently due to avoidance of antidepressant and neuroleptic medication while continuing to take lithium, or in some cases, avoidance of

all medications.

Several studies focused on childbirth and child-rearing in families where one parent is manic-depressive. We found that manic-depressive fathers appeared to be vulnerable to development of affective, primarily manic, episodes during their wifes' pregnancies, compared to afterwards. Special attention to prevention during this period has been recommended.

Using videotapes and blind raters, we have detected differences in high-risk infants compared with normal controls. Children of bipolar parents tended to show extremes in their responses to the pain and sorrow of others and were either unusually empathic or avoided the distress of others. Such children were more disruptive in background climates of anger and conflict, and in the home they displayed more frequent and severe behavioral problems than normal controls. Child-rearing practices and attitudes of bipolar mothers differed from controls in several respects. They were less likely to encourage openness to experience in their children and were overprotective; they were perceived to be more disorganized and less active in interaction with their children.

A study on the effects on patients of a shift of interdisciplinary roles on our inpatient research ward showed that emphasizing the role of primary nurse clinicians, de-emphasizing the role of individual therapy and management by physicians, and increasing the number, strenuousness and importance of research procedures was associated with a precipitous decline in the number of against-medical-advice discharges. A factor in this improvement seemed to be the increasing involvement and investment of the patient as a collaborator in the research and a shift in emphasis in the ward philosophy from psychological to biological causes of affective illness.

Brief outpatient studies of depressive syndromes and character disorders expanded our efforts to apply psychobiological research strategies across a broader spectrum of psychiatric disorders. Sixteen patients with depression-spectrum illnesses were studied with circadian rhythm monitoring (activity, temperature, and sleep), neuroendocrine testing (protirelin infusions and 0.5 mg dexamethasone tests), and neurotransmitter studies (cerebrospinal fluid, blood, and urine). In addition, the first patients were entered into a new program of research on rejection-sensitive dysphoria, which seeks to explore possible limbic system dysfunction in this disorder characterized by affective and behavioral dyscontrol. Finally, small scale treatment studies of treatment-refractory rapid-cycling bipolar patients were carried on.

Major findings this year include:

Global endogenicity ratings correlate with dexamethasone test abnormalities and early temperature minima.

Dexamethasone test abnormalities did not predict response to antidepressants. Initially abnormal results did, however, normalize if a clinical response occurred.

Abnormal dexamethasone test results correlated with blunted TSH responses to

protirelin.

Some borderline patients with rejection-sensitive dysphoria and symptoms suggestive of complex partial seizures respond to carbamazepine.

Two of five treatment-unresponsive rapid-cycling bipolar patients stopped cycling when treated with therapeutic doses of a monoamine oxidase inhibitor.

Basic and clinical studies of the neuronal and glial enolases have continued this year under Dr. Paul J. Marangos. Previous immunohistochemical studies established that one form of enolase is strictly localized in neurons and neuroendocrine cells and it has been named Neuron Specific Enolase or NSE. Another enolase isoenzyme has also been isolated and shown to be localized in glial cells and has been designated Non-Neuronal Enolase or NNE.

Our research efforts have focused on the use of NNE and NSE as probes of CNS morphology, pathology and development as markers of the diffuse neuroendocrine (APUD) system.

Important findings this past year include:

In collaboration with Drs. Desmond Carney, John Minna and Adi Gazdar (NCI) Dr. Marangos found that NSE levels are a clear biochemical marker of the presence and severity of small lung cell carcinomas. In vitro studies indicate that the source of plasma NSE is probably the cancer cells themselves.

NSE has been shown to be a biochemical marker of other APUDoma carcinomas as well. In collaboration with Dr. Richard Prinz at Loyola University Dr. Marangos found that serum NSE levels were elevated in patients with non-functioning pancreatic islet cell carcinoma.

In collaboration with Drs. Robert Seeger (UCLA) and Paul Zeltzer (U of Texas) NSE levels were found to indicate the presence of neuroblastoma and to be correlated with survival times. Thus NSE can be used to detect certain carcinomas, to assess responses to treatment and to predict clinical outcome.

Developmental studies with Drs. Whitehead and Maxwell at the University of Connecticut have shown that the appearance of NSE in developing neurons is related to the appearance of synapses and functional activity.

The demonstration of pharmacologically relevant binding sites for the benzodiazepines in brain suggest that endogenous ligands for the brain benzodiazepine receptor may exist. Studies during the past year have focused on the characterization of the benzodiazepine receptor using newly available ligands.

Important findings during the past year include the following:

Using the newly available "peripheral" type benzodiazepine receptor ligands [^{3}H] RO-5-4864 we have shown that peripheral type receptors exist in brain

and constitute 25% of the central binding sites. Additional studies indicate that the peripheral type benzodiazepine receptor, in contrast to the central type, is not coupled to the GABA receptor-chloride ionophore complex.

Benzodiazepine agonists and antagonists interact differently with the peripheral type receptor. The antagonist β-carbolines' binding to brain membranes, unlike agonists, is unaffected by GABA.

Preliminary studies indicate that benzodiazepines inhibit calmodulin-induced phosphorylation of brain proteins. Since protein phosphorylation is one of the major mechanisms of macromolecular modulation in brain, this effect of benzodiazepines may be an important mechanism of their action.

The role of adenosine and adenosine receptors in the central nervous system has been investigated by Dr. Marangos and his co-workers. There is considerable evidence that adenosine functions as a neurotransmitter that modulates the action of other neurotransmitters in the brain and thereby inhibits neuronal activity.

Significant findings this year include:

Using 2 stable analogues of adenosine, Dr. Patel has identified two binding sites in rat brain synaptosomal membranes. Autoradiographic studies showed these binding sites are probably located in the granule cell layer of cerebellar cortex, hippocampus and the superficial layer of the superior colliculus.

Behavioral studies in collaboration with Dr. Jacqueline Crawley showed that the adenosine analogues are potent sedatives. Chronic caffeine administration increases both adenosine and benzodiazepine receptor number and is probably an antagonist in both systems.

We have identified an adenosine reuptake site in brain that is distinct from the adenosine receptor. Adenosine reuptake blockade lowers the sedation threshold for exogenous adenosine.

Other studies indicate that the calcium ionophore may be coupled to the adenosine receptor.

Dr. Steven Paul and his associates continue to conduct basic investigations of receptors for neurotransmitters, neuropeptides, and neuromodulators in the CNS. High affinity, stereospecific recognition sites (receptors) for putative neurotransmitters, neuromodulators and many psychotherapeutic agents have been identified in the mammalian central nervous system. The interaction of neurotransmitter/neuromodulators with these sites is believed to initiate a series of events that leads to a behavioral/physiological response. The interaction of drugs with these sites is believed to lead to pharmacologic responses. The existence of stereospecific binding sites for psychotropic drugs suggests that endogenous modulators exist that mimic or antagonize the actions of the drugs. Currently several receptor systems are under study.

Significant findings this year include:

A series of studies of binding properties of the benzodiazepine receptors which are summarized in Z01 MH 01836-04 CP have led to the concept that benzodiazepine agonists and antagonists bind to separate subsites or "domains" on the benzodiazepine receptor. Studies with β-carboline benzodiazepine antagonists indicate that occupation of either an agonist or antagonist domain may be sufficient to interrupt or prevent motor seizures in genetically susceptible mice.

Results of studies of the behavioral effects of benzodiazepine antagonists provide the first evidence that sedative properties of benzodiazepines depend on their interaction with the benzodiazepine receptor, and that this receptor is involved in the physiological regulation of sleep. These studies also indicate that benzodiazepine antagonists could be useful as therapeutic agents in disease states associated with excessive sleepiness.

Preliminary studies indicate that benzodiazepine antagonists may be anxiogenic in the primate.

Behavioral studies indicate that adenosine and its analogues induce a paradoxical state of behavioral sedation and EEG arousal, termed "quiescent waking." This effect appears to depend on central mechanisms, not peripheral changes in blood pressure induced by these agents.

Previous studies demonstrated that [^{3}H] imipramine labels a "serotonin transporter" (recognition site + transport protein) in brain and platelet of both humans and rat. The number of these sites is genetically determined and appears to be reduced in depressed patients regardless of clinical state. Studies currently underway are designed to isolate an in vitro serotonin recognition system in artificial membranes.

Recognition sites for amphetamine have been identified in rat hypothalamus and striatum, and may be a locus of action for the appetite suppressing properties of these drugs.

Localization of cholecystokinin (CCK) receptors has been carried out in brain. CCK receptors are concentrated on neuronal elements in the caudate nucleus. The number of CCK receptor sites is markedly reduced in patients with Huntington's disease, in the caudate and in the cerebral cortex.

SUMMARY

The Clinical Psychobiology Branch has been extensively reorganized. The Chief, Dr. Goodwin, and several senior investigators have left the Branch to take on new responsibilities elsewhere. The Branch will continue to focus its research on the affective disorders; a major theme of this research will be sleep and circadian rhythms. This work will gain new impetus from the merger of the sleep laboratory and its personnel with the Branch. With these resources we have established a facility in which patients' and healthy volunteers' sleep and circadian rhythms can be monitored in an environment from which 24-hour time cues have been excluded. Clinical studies will be complemented by basic research in the neurophysiology and neuropharmacology of circadian rhythms and sleep under the direction of Drs. Gerard Groos and Wallace Mendelson, who joined the Branch this year.

The clinical research of the Branch has progressed from descriptive studies of circadian rhythms in patients to experimental manipulations of the timing of sleeping and waking and light and dark. Results of these experiments have led to two novel non-pharmacological treatments of depression.

Laboratory experiments have confirmed that antidepressant drugs alter the timing of circadian clocks or pacemakers. Future research will explore underlying mechanisms of this pharmacological link between circadian rhythms and affective disorders.

Our clinical research has benefitted greatly from a more efficient use of patient beds on the inpatient unit. We have greatly expanded outpatient studies from which patients can be admitted to the unit for one week or less for intensive inpatient research studies. With this arrangement two to three new patients per week have been admitted to the ward. We have also made much more efficient use of beds by admitting normal volunteers for brief periods to participate in specific studies, rather than resident normal volunteers who remain on the unit for three months.

Annual Report of the Laboratory of Clinical Science
National Institute of Mental Health
Irwin J. Kopin, M.D., Chief
October 1, 1981 through September 30, 1982

Introduction

As in the past, the various Sections and Units of the Laboratory of Clinical Science have conducted independent programs of research and appropriate collaborations within the Laboratory and with investigators in several other institutes as well as with others outside of the NIH. The major activities of the programs are directed toward defining mechanisms regulating the development and function of the nervous system, understanding the role of the nervous system in maintaining bodily functions, internal environment, and mental state and determining the molecular events underlying drug and hormonal actions. Clinical studies are aimed not only at defining abnormalities but at explaining mechanisms of disturbed brain or other neurological functions to provide rational bases for approaches to therapy of these disorders.

Although there have been leadership changes in the Intramural Research Program and the administrative structure of the laboratory will be changing to allow development of new areas and to recognize growth of younger members of the staff by promotion into positions appropriate to their degree of scientific stature, the research program has proceeded undisturbed and continues to evolve in accordance with the most promising directions as preceived by the individual investigators. A portion of the laboratory will be included in a new laboratory under the direction of Dr. Michael Brownstein. The new laboratory will consist of Drs. Martin Zatz, Miklos Palkovits and Fusao Hirata as Section Chiefs. Appropriate resources have been allocated for the creation of this new laboratory and to allow for professional development of several of the younger members of its staff.

Dr. Michael Ebert, Chief of the Section on Experimental Therapeutics has been appointed Clinical Director, NIMH and will continue in a dual role.

Dr. Judith Rapoport will be joining the Laboratory as Chief, Section on Child Psychiatry. Dr. Irwin Kopin, Chief of the Laboratory of Clinical Science will also serve as Associate Director for Clinical Research in the Intramural Research Program. These and several other administrative changes and space allocations will serve to encourage scientific interactions among clinical investigators and basic scientists.

The following summaries of the year's activities have been provided by the Unit and Section Chiefs of the Laboratory as presently constituted.

Section on Pharmacology
Julius Axelrod, Ph.D., Chief

The possible function of carboxyl methylation of membrane proteins was studied in neuroblastoma cell line NIE-115. These cells were found to be rich in the enzyme protein carboxyl methylase (PCM). The enzyme is mainly localized

in the cytosol while its substrates, the methyl acceptor proteins are present largely in the particulate fraction. These data suggest that PCM functions as a regulator of membrane bound proteins. Indeed, in the NIE-115 cells we found an increase in enzyme activity (two-fold) and a dramatic increase in methyl acceptor proteins (five-fold) as the cells undergo morphological and functional differentiation. The time course of this increase in carboxyl methylation paralleled the development of electrophysiological response of these cells. These data show a close relationship between the development of carboxyl methylation and membrane ion translocation. Protein carboxyl methylation has also been associated with exocytotic release. The latter being dependent on depolarization of the cells' membrane and Ca++ ions movements.

In the posterior pituitary lobe, exocytotic release of vasopressin, oxytocin and their corresponding neurophysins occur upon depolarization. We have studied carboxyl methylation of proteins in posterior pituitary lobes of Brattleboro rats with diabetes insipidus who lack vasopressin and vasopressin-neurophysin. In these rats methyl acceptor protein capacity was found to be 80% lower than in control rats while PCM activity is about 40% higher. The low methyl acceptor protein capacity is due to the low methylation of 11K daltons protein reflecting the absence of vasopressin-neurophysin which is a major substrate in the posterior pituitary. The increased enzyme activity can be attributed to the hyperactivity of the posterior pituitary of the Brattleboro rat.

The newly discovered methylation of free fatty acids by S-adenosyl-methionine was characterized in human red blood cells. The reaction occurs in whole cells on the cytoplasmic side of the plasma membrane. Biosynthesis of fatty acid methyl esters is one of the consequences of phospholipase A_2 activation in plasma membranes. The biosynthesis of another nonpolar methylated product was discovered upon incubation of lung membranes with oleoylcoenzyme A and S-adenosyl-methionine. This product was identified as S-methyl-oleoylcysteamide and appears to be formed by cell membranes, including plasma membranes, after amidase cleavage of oleoylcoenzyme A.

The mouse pituitary tumor AtT-20/D16-16 is a cell line rich in corticotrophs and is an excellent model to study the physiological regulation of synthesis, storage and secretion of ACTH and beta-endorphin. Initial studies focussed on the characterization of the secretory response to stimulants such as the recently available synthetic 41-residue corticotropin-releasing factor (CRF) and to antagonists such as glucocorticoids. CRF caused a marked increase in the release of ACTH and beta-endorphin in AtT-20 cells. CRF was also found to stimulate phospholipid methylation, a reaction which appears in other cell systems as an important transducing mechanism following hormone-receptor interaction. Post-translational modification of protein by carboxyl methylation has been implicated in exocytotic secretion in both endocrine and exocrine glands and similar operative mechanisms stimulated by CRF were found in the AtT-20 cell. Secretion of ACTH and beta-endorphin from AtT-20 cells appears to be under multifactorial regulation by both peptides and neurotransmitters. We have identified beta-adrenergic receptors in the AtT-20 cells by both binding and ACTH secretory studies and have also found that some CNS peptides such as VIP and somatostatin

influence the secretory response to ACTH. These observations of hormonal interaction will enable us to further dissect both cellular and molecular mechanisms involved in the transduction of the physiological response in the AtT-20 cells and will eventually provide insight and understanding of the importance of the anterior pituitary in the pathogenesis of stress.

The avian pineal gland contains a circadian oscillator and a photo-receptor which regulate the synthesis and secretion of the hormone melatonin. Cultured chicken pineal glands express circadian rhythms in serotonin N-acetyltransferase activity and in cyclic AMP and cyclic GMP levels. Light exposure of the gland during the subjective night reduces the levels of both nucleotides and, subsequently, the activity of serotonin N-acetyltransferase. The effects of pharmacological agents which selectively affect cyclic AMP or cyclic GMP indicate that it is cyclic AMP levels which mediate the effects of light and regulate enzyme activity.

The eye of Aplysia expresses a robust circadian rhythm of neural output in vitro. Serotonin and forskolin both stimulate adenylate cyclase activity in homogenates. Their effects on the circadian pacemaker, as reflected in the phase response curves produced in intact eyes, are identical. Thus serotonin acts on the circadian pacemaker via its activation of adenylate cyclase.

We have previously shown that many receptors interact with methyltransferase of phospholipids and that stimulation of receptors result in increase of phospholipid methylation. To establish such interaction, beta-adrenergic receptors were partially purified and transplanted into human red blood cell ghosts which have the methyltransferases but not beta-adrenergic receptors. The ghost membranes responded to beta-agonists as measured by stimulation of phospholipid methylation. The idea that stimulation of phospholipid methylation by ligands requires receptors has been further established by the findings that anti-IgE receptor antibody can increase phospholipid methylation in plasma membrane fraction but not in mitochondrial fraction from rat mast cells, although both fractions have the methyltransferase activities. IgE receptors are primarily located in the plasma membranes.

A phospholipase inhibitory protein has been isolated from the conditioned media of rabbit neurophils incubated with glucocorticoids. Purified lipomodulin can inhibit arachidonate release from fibroblast stimulated by bradykinin (hormone) from lymphocytes stimulated by Con A (mitogen) and from neutrophils stimulated by fMet-Leu-Phe (chemoattractant). The inhibition of arachidonate release from these cells can be observed by the treatment with glucocorticoids such as dexamethasone. The monoclonal antibody against lipomodulin reversed the effects of both lipomodulin and glucocorticoids. These results suggest that lipomodulin mimics the action of glucocorticoid on the suppression of arachidonate release from various cells.

Central catecholamines, histamine, serotonin and several neuropeptides are involved in the regulation of spontaneous and sodium-sensitive genetic hypertension. There are specific and selective changes in catecholamine metabolism in hypothalamic and brain stem nuclei in genetic sodium dependent hypertension

in the rat, and in sodium depleted dogs. There are large changes in catecholamine metabolism in the heart and adrenal medulla of genetic, sodium sensitive hypertensive rats. Specific binding of prostaglandin E_2 has been described in rat brain membranes.

Angiotensin-converting enzyme (ACE) activity is heterogeneously distributed in the rat brain, with highest activity in the subfornical organ. Spontaneously hypertensive rats show specific changes in ACE in intermediate and anterior pituitary lobes, and in specific nuclei of the brain stem. Changes in somatostatin occur in the pituitary and selective hypothalamic nuclei of rats lacking vasopressin. There are alterations in protein carboxyl methylation in rats lacking vasopressin.

We plan to study further the physiological and pathophysiological interactions between the vasopressin and angiotensin systems in brain and pituitary gland, and the interrelations between vasopressin, somatostatin, opioid peptides and biogenic amines in the posterior and intermediate lobes of the pituitary gland.

Section on Histopharmacology
David M. Jacobowitz, Ph.D., Chief

This section has been involved with studies of peptidergic, monoaminergic and cholinergic neurons in the central nervous system. Immunocytochemical and neurochemical characterization of peptide-containing nerves in the brain and periphery has been pursued. Histochemical and biochemical analyses are carried out at the level of discrete central nuclei and pathways in order to define the underlying basis for behavioral changes.

Highly specific and sensitive radioimmunoassays coupled to HPLC and gel filtration techniques were utilized to identify secretin, motilin and pancreatic polypeptide immunoreactivity in the brain of the rat. Immunocytochemical studies revealed a wide distribution of pancreatic polypeptide (PP) immunoreactive cell bodies and nerve fibers in the rat central and peripheral nervous systems. A number of PP-immunoreactive cells were demonstrated to coexist with catecholamine neurons in the pons-medulla and sympathetic ganglia. Motilin-like immunoreactive nerve fibers and cell bodies were observed in the hypothalamus, preoptic areas and Purkinje cells of the cerebellum. Radioimmunological studies revealed a wide distribution pattern of secretin in the brain of the rat. A brain-pituitary pathway for secretin has also been identified. Intraventricular injection of secretin reduced open field activity and the number of novel-object approaches in rats. Secretin also decreased respiration rate in anesthetized rats and increased defecation in awake rats. Intravenous injection of secretin in anesthetized hydrated rats caused a dose-related effect (Drs. O'Donohue, Olschowka and Mr. Charlton).

Previous studies demonstrated that acetylated and deacetylated forms of β-endorphin exist in the same neurons and pituitary cells in rat and human brain. α-MSH released from the neurons may influence processes of attention, arousal and learning; β-endorphin released from the neurons may influence analgesia.

The acetylated form of α-MSH is 2-3 orders of magnitude more potent than deacetylated α-MSH, while the acetylated form of β-endorphin is 3-4-fold less potent than β-endorphin. Enzymes which acetylated α-MSH and β-endorphin have been identified in brain and pituitary. Among these is a general acetyltransferase (GAT) present in all organs of the rat. In addition, a specific enzyme capable of acetylating both opiate (β-endorphin) and melanotropic (α-MSH) peptides has been identified, localized to secretory vessicles and named opiomelanotropin acetyltransferase (OMAT). OMAT activity can be induced by physiological manipulation which induces MSH synthesis.

Studies of the physiology and pharmacology of α-MSH in the brain have also continued. It was found that α-MSH administration selectively influences visual but not auditory learning. Furthermore, a compound 4-Norleucine, 7-D-Phenylalanine-α-MSH has been synthesized and been found to have identical effects on arousal as α-MSH but opposite effects on learning - suggesting multiple α-MSH receptors in brain. In addition, α-MSH injected into the dorsomedial nucleus of the hypothalamus has been found to cause increases in heart rate. The results of these studies demonstrate that brain α-MSH may be involved in a variety of of different physiological functions.

We have previously reported that a population of afferent nerves from the arterial baroreceptors contain the neuropeptide substance P (SP). These nerves were traced to the nucleus of the tractus diagonalis (NTS) of the medulla oblongata of the hindbrain. Several studies have focused attention on the possible pathogenesis of hypertension by central catecholamine alterations in spontaneously hypertensive (SHR) rats. The present experiment deals with two substrains of the Sabra rat, one of which is genetically predisposed to develop hypertension when fed on a DOCA salt diet (SBH), while the other is hypertension resistant (SBN). In the present experiment, SP, catecholamine and vasopressin were studied in the NTS of these rats. Higher concentrations of NE, Epi were observed in the NTS of the SBH strain as compared to the control (SB) or hypertension resistant strain (SBN). The SB was significantly greater than the SBN. SP levels were significantly higher in the SBH and SBN as compared with the SB strain. Vasopressin was significantly higher in SBH and lower in SBN than SB. The significance of a similar directional change for SP in the SBH and SBN strain is unknown. The changes in catecholamines and vasopressin do, however, correlate with the predisposition for hypertension. Whether the changes observed in the catecholamine and peptide systems are causitive or adaptive phenomena in the pathogenesis of hypertension susceptibility or resistance awaits further investigation.

There exists a system of dense acetylcholinesterase (ACHE) staining cholinergic cells in the rat forebrain which has been designated as the magnocellular nuclei of the basal forebrain (MNBF). Because of the diffuseness of the localization of the MNBF, no single lesion of this cell system can reveal the extent of the cholinergic projections throughout the brain. Therefore, a parceling of this chain into segments that are appropriate for stereotaxic lesions seems most feasible. This study was undertaken to elucidate the cholinergic projections emanating from a specific portion of the MNBF, the ventral aspect of the nucleus of the tractus diagonalis (td). Following lesions

choline acetyltransferase (ChAT) was used as the marker to determine cholinergic nerve projection sites. Significant decreases in ChAT activity were observed in the cingulate, frontal and occipital cortices, the hippocampus and dentate gyrus. The nucleus of the tractus diagonalis has been revealed to be a major cholinergic nucleus innervating many parts of the cortex and basal forebrain structures. This fact, together with the presence of a mesolimbic dopaminergic input (reported by this laboratory last year) from the A10 dopamine cell body area, underlines the potential physiological significance of a dopaminergic/cholinergic interaction at the level of the A10/td system which could have important influences on emotional behavior via connections with the cortex and limbic system. The potential for interaction between dopaminergic and the cholinergic system may prove useful in the future in understanding the neuroanatomical basis for the mechanisms of action of the neuroleptics and other drugs which interact with dopaminergic neurons.

Section on Medicine
Irwin J. Kopin, M.D., Chief

Research projects in the Section on Medicine continue to focus on neurotransmitter mechanisms in the central and peripheral sympathetic nervous systems and the interactions of drugs, hormones, and disease processes with these regulatory processes.

Fluorocatecholamine derivatives have been studied to determine their potential usefulness as clinical tools to be labelled with ^{18}F for NMR or PET scanning. 6-Fluorodopamine has been found to be taken up into sympathetic nerves and converted to 6-fluoronorepinephrine, which is stored and released during sympathetic nerve stimulation along with endogenous norepinephrine, indicating its usefulness as an *in vivo* marker for sympathetic function.

Studies in pithed rats have continued as a means for rigorously controlling sympathetic outflow during which we measure norepinephrine release into plasma and monitor the pressor effects of stimulation. Rats made hypertensive by treatment with DOCA and salt or by renal compression have increased responses to stimulation with relatively less norepinephrine released, suggesting enhanced α-receptor sensitivity. Intrajunctional location of α_1-adrenoceptors and extrajunctional sites of α_2-adrenoceptors in vascular smooth muscle have been further examined. Uptake blockade with DMI or cocaine selectively enhances responses to administered norepinephrine at α_1-adrenoceptors whereas the responses to stimulation are not enhanced because potentiation of norepinephrine at presynaptic α_2-adrenoceptors prevents release of norepinephrine. The uptake site appears to be functionally or anatomically located between the intrajunctional (α_1-) adrenoceptors and extrajunctional (α_2-) adrenoceptors.

A technique has been developed for estimating the synaptic concentration of norepinephrine at the sympathetic vascular smooth muscle neuroeffector junction e.g., a synaptic concentration of 7 nM evokes a 50 mmHg in blood pressure. The norepinephrine concentration - pressor response curve is remarkably consistent with the kinetics previously reported *in vitro*. DMI-sensitive uptake of norepinephrine into sympathetic nerves appears to be responsible for removal of

55% of the norepinephrine as it passes from the junctional gap into the circulation.

Studies in intact rats complement those in pithed animals to reveal processes which are regulated by the central nervous system. One project involves microinjection of substances directly into specific brain areas or direct examination of neurotransmitter related substances in brain areas using biochemical or immunohistological techniques.

A series of studies of effects of direct injection of prostaglandins into brain areas have shown that $PGP_{2\alpha}$-injected into the paraventricular, dorsomedial, or posterior hypothalamic nuclei evokes sympathetic discharge and affects respiration and body temperature. Opiates injected into the anterior hypothalamic in low doses evoke naloxone-reversible increases in heart rate, blood pressure and plasma catecholamines whereas higher doses produce noloxone-reversible indirect cardiac depression via vagal stimulation as well as by respiratory depression.

Chronic noloxone treatment increases opiate receptor number and enhances cardiovascular, but not respiratory depressant effects of morphine. Dopamine receptors are increased in diabetic rats because of chronic hyperglycemia inhibition of dopaminergic neurones. This is attended by increased sensitivity to receptor blockade by haloperidol.

Vasopressin, the antidiuretic hormone secreted from the posterior pituitary, has other functions in brain, where its neurotransmitter function appears to be controlled independently from osmotic pressure. Vasopressin levels are elevated in a strain of hypertension-prone (Sabra) rats, but it is not known if this is a primary or compensatory effect.

Using immunohistological methods and an antibody to Glutamic Acid Decarboxylase (GAD), neurones which use α-aminobutyric acid (GABA) as a neurotransmitter have been localized to specific neurones at both light- and electron-microscopic levels. This enzyme has been definitively distinguished from cysteine decarboxylase which also decarboxylates glutamic acid and is present in liver and non-neuronal cells in brain. A double-staining method to simultaneously visualize GAD and somotostatin has shown that these two substances are present in the same neurones in brain.

Responses of intact animals provide the next link in the bridge from basic to clinical studies. Hemorrhage evokes several compensatory responses involving the sympathetic, vasopressin, and renin-angiotensin systems. Prostaglandins enhance these responses and facilitate recovery, whereas vasopressin-deficient Brattleboro rats have blunted compensatory hemorrhagic responses and are unable to compensate when sympatho-adrenal medullary and renin-angiotensin responses are blocked.

Chronic treatment of SHR rats with arachidonic acid, the prostaglandin precursor, lowers blood pressure. The metabolism of arachidate in SHR rats appears to differ from that in WKY rats because the effect is not seen in these normotensive rats and is blocked in SHR rats by indomethacin treatment.

In humans, plasma levels of norepinephrine are a result of overflow of the transmitter from sympathetic neuronal synapses. The net overflow is altered by processes for removal (uptake and metabolism) of the released amine prior to entry into the circulation. The plasma levels of norepinephrine are also modulated by its removal from the circulation. Studies with isotopically-labelled norepinephrine have shown that in patients with elevated levels of norepinephrine the increase appears to be due to enhanced overflow of norepinephrine into plasma rather than decreased removal of the catecholamine. During cardiac bypass operations, anesthesia diminishes and the stress of the incision elevates plasma norepinephrine levels. After completion of the operation, the heart begins to take up norepinephrine from the circulation whereas the peripheral sympathetic nerves release large amounts of the amine.

The metabolites of norepinephrine in urine reflect its total synthesis in the body, but their relative quantities reflects the degree of peripheral sympathetic activity. Normetanephrine is formed from norepinephrine which reaches the circulation or is metabolized at non-neuronal sites, whereas the deaminated metabolites reflect intraneuronal metabolism. Patients with isolated orthostatic hypotension (IOH) due to disordered peripheral sympathetic nerves have proportionate decreases in urinary normetanephrine, 3-methoxy-4-hydroxy-mandelic acid (VMA) and 3-methoxy-4-hydroxy phenyl glycol (MHPG). Patients with central nervous system dysfunction due to multiple system atrophy (MSA) have intact peripheral sympathetic neurones which are not activated appropriately. This results in a disproportionate decrease in normetanephrine excretion with only a slight decrease in total catecholamine metabolites.

The inappropriate sympathetic function in these patients is reflected in pineal function as well. The IOH patients (with a peripheral sympathetic disorder) have low 6-hydroxymelatonin excretion in urine, but the circadian pattern is maintained, with most of the pineal hormone metabolite excreted at night. Many patients with MSA, however, have normal 6-hydroxymelatonin excretion, but the circadian pattern is disturbed, with daytime levels of the metabolite equal or higher than night-time excretion rates.

In the various forms of orthostatic hypotension, parasympathetic as well as sympathetic function appears disturbed. A battery of tests is being devised for early diagnosis of these disorders because impotence is frequently the first symptom of the disorder and, in the absence of other evidence of neurological dysfunction is misdiagnosed as having a psychogenic basis.

Section on Experimental Therapeutics
Michael H. Ebert, M.D., Chief

Clinical research in the Section on Experimental Therapeutics has focused this year on neurochemical, neuroendocrine, and behavioral studies of anorexia nervosa, Korsakoff's psychosis, and several neurological diseases with abnormalities in catecholamine metabolism. Unfortunately the laboratory work in the Section was compromised by the ADAMHA hiring freeze which eventually eliminated the support personnel in the Section.

A collaborative research effort with Dr. Carl Merril of LGCB, NIMH has continued this year, and the laboratory supporting this research has continued to received support from the NIAAA. In these studies, two-dimensional electrophoresis (2DE) has been developed into a quantitative tool for the identification of molecular markers and probes for neuropsychiatric diseases. To perform this type of analysis more than 1,000 cellular proteins or a somewhat smaller number of proteins in a biological fluid are resolved on the two-dimensional electrophoretogram, visualized by staining or autoradiography, and then measured for variation in spot density or position with the aid of a computer.

A major effort was made in the past year to further improve this technology in several areas. The ultrasensitive silver stain for proteins developed by Dr. Merril has been simplified and shown to possess sufficient linearity and reproducibility for quantitative use. The silver stain has also been shown to be effective for detecting trace amounts of DNA, RNA, and lipopolysaccharides.

In collaboration with Wayne Rasband of RSB, NIMH, the computer analysis of electrophoretic patterns has been refined using an RSB PDP 11/60 computer so that measurements are made more accurately and quickly. A larger computer, a VAX-750, is now being programmed for gel analysis with the assistance of Dr. Mark Miller of NCI.

Lesch-Nyhan syndrome is an X-linked recessive disease caused by a deficiency of hypoxanthine-guanine phosphoribosyltransferase. It results in spasticity, hyperuricemia, and self-mutilation. Quantitative differences in the activities of several peripheral cellular enzymes have been shown to be associated with it. One category of protein variation which might be detected with 2DE is a pattern of protein modulations secondary to a primary metabolic disturbance. In a study of Lesch-Nyhan patients, 400 lymphocyte proteins from each individual were measured and 11 proteins were identified that were consistently modulated with the disease.

Another category of polypeptide variation which may be observed with 2DE is the association of normal polymorphic protein variants with a disease locus within a pedigree (because of genetic linkage and a low rate of recombination) or within the population (because of linkage disequilibrium). A study on Huntington's disease (HD) was carried out this year to search for genetic markers of the disease. The study was done on 28 individuals, including 13 with HD, 2 at rish for HD, and neurological controls. A quantitative analysis of the 306 most dense lymphocyte proteins failed to show any correlation with HD. This result supports recent studies which have failed to confirm earlier reports of membrane and other abnormalities in the peripheral cells of individuals with HD. In the course of this study, a number of polymorphisms were discovered. For six polymorphic proteins, three phenotypes were observed in the population we studied. Our mathematical analysis indicates that the number of polymorphisms available by 2DE will make significant impact on linkage analysis.

Neurochemical and neuropharmacological studies of Alzheimer's dementia are concluding this year. A chronic treatment study of lecithin and an anticholinesterase (tetrahydroaminoacridine), administered in combination to increase acetylcholine turnover in the central nervous system, has not proved to be

efficacious in improving memory function in Alzheimer patients.

A genetic study of a Canadian family containing 45 members affected with Alzheimer's disease was completed this year. Ancestors were traced through 8 generations and 51 members were examined at the National Institute of Mental Health. Pedigree analysis indicated autosomal dominant inheritance. This family will be further studied with genetic marker techniques.

A study of the relationship between neurochemical changes within the central nervous system and memory function in Korsakoff's psychosis is underway. Although Korsakoff patients perform as poorly on recall tasks (episodic memory) as patients with early Alzheimer's disease, studies in collaboration with Dr. Weingartner (LPP, NIMH) demonstrate that different mechanisms underlie their memory failure. For example, Korsakoff patients are able to utilize cues such as the organization of material to be remembered in order to aid recall, since their ability to access knowledge structures in memory (semantic memory) is relatively intact compared to individuals with early Alzheimer's disease. Clinical assessment of patients with Korsakoff's psychosis indicate the presence of residual neurological signs several years after thiamine treatment of the acute (Wernicke) stage of the illness. Measurement of 6-hydroxy-melatonin in the urine of these patients (in collaboration with Drs. Higa and Markey) demonstrate substantial reduction in excretion of this major metabolite of melatonin. This biochemical finding suggests central adrenergic dysfunction in Korsakoff's psychosis. Analysis of CSF and plasma catecholamine metabolites are in progress, and neuropharmacological assessment of autonomic dysfunction is planned. An animal model of thiamine deficiency which neuropathologically resembles Korsakoff's dementia is being adapted to further investigate neurochemical abnormalities.

Studies of plasma and CSF kinetics of HVA (the major metabolite of dopamine) have continued using deuterium labelled HVA in an effort to develop a clinical method for measuring dopamine turnover in the brain, and provide an accurate physiological interpretation of HVA levels in lumbar CSF and plasma. Deuterated HVA and ^{14}C-insulin was injected into the lateral ventricle, lumbar subarachnoid space, or intravenously in rhesus macaque, and its appearance into the clearance from plasma, urine, and lumbar CSF was determined. The mechanisms of elimination of HVA from lumbar CSF and plasma, and the effect of probenecid on clearance from these compartments is being determined. The production rate of HVA is being compared in normal subjects and untreated patients with Parkinson's disease by analysis of the plasma and urinary excretion kinetics of intravenously administered deuterated HVA.

Clinical studies of anorexia nervosa have continued to focus on defining psychobiological and neurochemical changes that take place during the evolution of the chronic illness. A clinical design is utilized in which we study underweight anorexics, the same women after weight restoration, a separate group of women who were once underweight with anorexia but have been weight recovered for at least 20 months, and normal control women. Underweight anorexics have low CSF 5HIAA and HVA, indicating decreased brain serotonin and dopamine turnover that corrects with weight recovery. Likewise underweight anorexics have levels of

CSF total opioids that are several times higher than normal levels. In contrast, long-term weight recovered anorexics continue to have disturbances in central and peripheral norepinephrine (NE) metabolism compared to normals (decreased plasma and CSF NE and NE metabolites). This abnormality may bear some relationship to chronic changes in appetite and metabolic regulation.

Unit on Neuroendocrinology
Michael J. Brownstein, M.D., Chief

The members of this Unit have interests that span the entire field of neurobiology. Projects range from basic anatomy to molecular genetics, from behavior to cell biology.

Dr. Sherwood and her colleagues have succeeded in isolating and purifying to homogeneity the GnRH-like peptide responsible for spawning in the salmon. This substance differs from mammalian GnRH in two positions. Two other GnRH-like peptides have been detected in extracts of chicken brain. These peptides are in the process of being purified.

Work has continued on isolating the factor or factors elaborated by mouse fibrosarcomas that inhibit macrophage chemotaxis. It seems that tumor extracts contain more than one active agent; the pathophysiological importance of these agents remains to be seen.

The distributions of several neuropeptides (including VIP, substance P, somatostatin, and cholecystokinin) among central nuclei have been studied in detail, and the effect of lesions on peptide levels have been determined. These studies have allowed Dr. Palkovits and his coworkers to sketch the major peptidergic systems in the CNS. Detailed pictures of these systems have been obtained at the light microscopic and electron microscopic levels.

The biosynthesis of biologically active peptides continues to be an area of interest. The structure of the human preproenkephalin molecule has been elucidated by means of cloning and characterizing DNA complementary to its mRNA. Characterization of other molecules of biological importance is in progress. Processing and intracellular translocation of peptide precursors is being investigated. Drs. Pruss and Eiden are preparing monoclonal antibodies against chromaffin granules to help catalogue the proteins that form their walls and comprise their contents. This work will lay the foundation for future studies of granule assembly and precursor packaging.

The precursors seem to be packaged in the granule along with enzymes that are responsible for liberating active peptides. One such processing enzyme has been detected and partially characterized in chromaffin granules.

A method has been developed for purifying pineal serotonin N-acetyltransferase. If antibodies can be raised against this enzyme, which limits the rate of melatonin production, regulation of its activity will be studied in detail.

Unit on Pharamcological Applications of Mass Spectrometry
Dr. Sanford P. Markey, Ph.D., Chief

In collaboration with Dr. Fred Abramson a method has been developed for detecting specific nuclides (^{13}C, ^{14}C, ^{32}S, ^{14}N ^{15}N, etc.) as compounds emerge from capillary gas chromatographic columns. A low-pressure microwave discharge plasma completely combusts eluting carbonaceous compounds and converts them to di- to triatomic molecules which can be readily measured by low resolution mass spectrometric methods. This system offers considerable advantages over existing methods for detecting and characterizing metabolites of labeled compounds or determining the elemental composition of unknown materials.

Studies on the use of urinary 6-hydroxymelatonin as an index of pineal gland production of melatonin have been continued by Dr. Markey. Working with Dr. Buell the absence of extrapineal production of circulating melatonin in rats was demonstrated, clarifying an issue which has muddied pineal physiology for some time. The longitudinal study of melatonin production during the pubertal development of girls has shown that each girl exhibits a consistent pattern of melatonin production. This pattern does not vary with season or advancement in pubertal stage. However, the girls are still in early stages of puberty and will continue to be studied in the next year. A study of the effects of the menstrual cycle on melatonin production by young women of childbearing age is being initiated. To serve as controls for these studies as well as answer questions regarding seasonal effects, adult males have been sampled over the past year and will be analyzed.

Dr. Higa has initiated a study of 5-methoxyindole acetic acid to determine if this compound is also exclusively of pineal origin, and whether it could be more simply measured than 6-hydroxymelatonin.

Dr. Wilfried Mayer has completed the synthesis of di-^{18}O labelled 3,4,-dihydroxyphenylethylene glycol, a norepinephrine metabolite, for use in studies on the disposition and fate of this metabolite in experimental animals.

Unit on Clinical Biochemistry
Mrs. Edna K. Gordon, Chief

The Unit on Clinical Biochemistry develops and applies new procedures for the quantitative analysis of biogenic amines and their metabolites in human body fluids and is involved in studies designed to evaluate the significance of changes in levels or turnover rates of these metabolites.

There is a popular concept that total urinary MHPG may reflect brain catecholamine metabolism, we have obtained good evidence which indicates that less than 30% of urinary MHPG is derived from brain. Methods using GC-MS have been developed for accurate assay of MHPG and its deuterated derivatives. Deuterated MHPG injected as a bolus or administered as a slow infusion over 3-4 hours is excreted in urine as a conjugate of MHPG or converted to VMA. Unconjugated plasma MHPG is a major transitional metabolite which accounts for about 2/3 of the total urinary catecholamine metabolites. About half of the MHPG in plasma is excreted as a conjugate whereas the remainder is converted to VMA and accounts for about

half of the VMA excreted in the urine. Both conjugated MHPG and VMA are derived from sources independent of plasma MHPG, e.g., DHPG.

Plasma MHPG levels may, however, provide a useful index of cummulative sympathetic activity in the whole body. The levels of MHPG in plasma are significantly greater in depressed patients who fail to suppress plasma cortisol levels in response to dexamethasone than in patients who show normal supression. There is an exchange of plasma and CSF MHPG so that plasma and CSF levels of free MHPG are highly correlated. CSF levels are always higher than those in plasma, even when large amounts of the catecholamine metabolite are derived from the tumor of the adrenal medulla. Plasma and CSF behave like a two-comparment system with similar rate constants for entry into and exit from the CSF compartment. MHPG formed, but not metabolized, in the central nervous system maintains CSF levels.

Methods for assay of urinary excretion of catecholamine metabolites have been developed and applied in a series of collaborative studies described in detail in other portions of this summary. Studies on Korsakoff's psychosis, orthostatic hypotension, anorexia nervosa, parkinson's disease, and anxiety - panic disorders are in progress. The effects of drugs such as clonidine and a new inhibitor of epinephrine synthesis have been examined and a collaborative study with Dr. LaBrosse of the University of Maryland Shock Trauma Unit involved determination of a patient with norepinephrine and dopamine metabolites from a cerebral ganglioneuroblastoma. We wish to determine which metabolites can be of help in identifying this type of tumor and which metabolite in blood plasma or urine can help in monitoring progress of patient or recurrence of tumor.

Annual Report of the Laboratory of Developmental Psychology

National Institute of Mental Health

October 1, 1981 - September 30, 1982

Marian Radke-Yarrow, Ph.D., Chief

Summary

Overview

The Laboratory of Developmental Psychology continues to dedicate its research efforts to studying the psychological and behavioral development of children in ways that (a) integrate investigation of normal and psychopathological development, and (b) attempt to understand the complex linkages, at all stages of development, among biomedical, experiential, and behavioral factors. These objectives are not unique to this Laboratory; however, what is unique is the study of these problems with a developmental perspective. Observations of relationships in the adult do not provide information on formative stages, developmental course, or potential early prevention or intervention. The study of children does not, in itself, yield such evidence. Research strategies are required that take advantage of sampling at different stages of development and that utilize longitudinal designs which permit the direct assessment of developmental changes. For obvious practical reasons, the latter designs can not often span the long period of years from infancy to adulthood. There is the fruitful alternative, however, of selecting critical longitudinal intervals (e.g. late infancy to early childhood, prepuberty through puberty) for investigating processes of formation, transition, and transformation in behavior, and for studying developmental changes in bio-behavioral relationships.

In the program of the Laboratory, these several strategies are used in pursuing a diversity of problems that fall into three major areas. Studies of bio-medical conditions of the child in relation to competent and disordered behavioral functioning are one core. A second focus is developmental psychopathology. Here our special concern is families in which there is parental psychopathology and in which we can investigate the interplay of the parents' illness and its expression in their rearing behavior and the children's development. Studies of pathology are always accompanied by investigations of normal families. The third focus is studies of certain primary response dimensions in normal and abnormal development; namely (a) the child's (and adult's) capacities for comprehending emotional and behavioral cues in other persons, (b) the child's affiliative and altruistic responses, and (c) the child's aggressive response patterns. Quite obviously, studies in these areas are a part of the work relating to the other two foci.

In the past several decades, much research in developmental psychology has painstakingly specialized in measures of children's cognition and in

mechanisms, such as reinforcement, for altering response frequencies of specified child behaviors. The processes underlying behavior have been studied somewhat segmentally. We have moved to an emphasis on understanding the interdependencies among various facets (cognitive, affective, and behavioral) of children's functioning. As a consequence, our conceptualizations, paradigms, and measurement approaches have changed materially. This brings us to issues of the kinds of research expertise required to carry out this research and to changes that have been made in Laboratory staffing to meet these needs. The research emphases described, the broadened view of competent and disordered functioning, and the combination of experimental and clinical research have made it necessary to bring together research skills that lie within several disciplines. The Laboratory now includes, in addition to developmental psychologists, a psychiatric social worker, a nurse, and child psychiatrists who work as teams on the major projects in the program.

The major projects are summarized below.

Summaries of Research Projects

Biomedical Conditions and Behavioral Functioning

In this area of research, studies of a variety of biomedical conditions have as their common research aim the assessment of correlated cognitive and behavioral functioning. One series of studies involves children with acute lymphocytic leukemia. Several years ago, Dr. Howard Moss reported findings of cognitive deficits in children who had been treated with cranial irradiation. Based on these findings, an extensive study has been undertaken by the National Cancer Institute, in which Dr. Moss is a collaborator. It involves child leukemia patients, in a number of medical centers across the country who are treated with one of two procedures: (a) CNS irradiation and intrathecal methotrexate or (b) a high dosage infusion of methotrexate, a treatment that hopefully is less neurotoxic than the CNS treatment. An extensive battery of tests (of attention, new learning, problem solving, immediate and delayed memory, general intelligence, motor functions, and behavior problems) is administered to the patients and to sibling controls, with the objectives of assessing the psychological and behavioral consequences of each treatment, and of identifying the specific areas and extent of deficit.

Two studies of chronic moderate undernutrition, (Drs. David Barrett and Marian Yarrow) reported in previous years, have been completed. Based on a Third World population and a United States population, undernutrition in the prenatal period and during the first two years was found to be associated with patterned behavioral characteristics of low responsivity to, and interest in, environmental stimulation, with low affiliativeness, and sad affect. Findings on sensori-motor and intellectual functioning varied in the two studies. In the Guatemalan sample, attentional deficits were impaired by undernutrition but not higher level cognitive

functioning. In the United States sample, undernutrition was associated with lower performance on tests of sensori-motor functioning, intelligence, and achievement.

A major new undertaking is a study of the behavioral accompaniments of pubertal changes. The interrelations of physical growth, endocrinological functioning, cognitive functioning, moods and emotions, and interpersonal behavior are investigated. Participants are 9- to 14-year olds and their parents who are seen at three periods of measurement, six months apart, providing data on timing and rate of both biological and behavioral changes. This work has required considerable preparation in the development and selection of appropriate testing procedures, and in the perfection of the longitudinal design. The study is being carried out collaboratively with endocrinologists in NICHD. Data from this project provide a normal sample for studies by NICHD of precocious and delayed puberty. Behavioral problems of adolescents are assessed across these samples, using the Achenbach Child Behavior Checklst, which was developed in this laboratory some years ago. Because this procedure yields measures standardized for age and sex, it is especially useful in comparing these samples. Dr. Elizabeth Susman is carrying the major responsibility for this project. Drs. Jerome Blue and Editha Nottelmann and Gale Inoff are coinvestigators. The first period of testing is just now underway.

In this work, Dr. Jerome Blue extends his other research with the Behavior Checklist. Using data collected earlier by Achenbach on a very large population of children, Dr. Blue has also directed his efforts to identifying behavior problems that are transient and those that are relatively stable across childhood. Behavior problems of 6- to 11-year olds and 12- to 16-year olds reported at intake at a child guidance clinic were compared with reports 6 and 18 months later. In both age groups, problem scales showing considerable stability over time ($r > .50$) are aggression, withdrawal, depression, hyperactivity, and delinquent behavior.

The transition from childhood to adolescence is investigated in another project, by Dr. Editha Nottelmann, in which prominence is given to environmental transitions that are imposed on children. The transition from elementary school to middle school or junior high school is a natural experiment of a comprehensive change in the child's environmental setting, involving altered conditions of supervision, peer networks, and expectations. This transition parallels pubertal changes. How children in early adolescence cope with these major biological and environmental discontinuities in their lives and what factors are associated with the development of behavior problems and competencies are the focii of analysis. Multiple measurements were made prior to and after school transition. More than 400 children have been studied. There is an inevitable lag between the end of data collection (which is now completed), the data reduction and processing (which is well underway) and the emergence of findings.

Studies of Aggression

Previous work in this Laboratory and elsewhere suggests the very early emergence (in the first two to three years) of individual patterns of affiliative and altruistic responding that remain fairly stable in qualitative characteristics through early childhood. Research on aggression also suggests that aggressive behaviors in later childhood have their roots in the early years. Drs. Mark Cummings, Carolyn Waxler, and Ronald Iannotti are carrying out studies on early manifestations of aggression. The aim is to identify significant parameters of early aggression. This has meant a search for dimensions or patterns of aggression that are characteristic of different developmental levels, that differentiate qualities of aggression, and that are signals of later serious aggressive behavior. Additionally, the relation of aggression to altruistic behavior and temperament is examined. Children in the second and third years of life have been studied in naturalistic and extensive laboratory observations with peers and adults over a 2-month period. They will be followed up at 4 years.

Findings of relatively stable individual differences in aggressive patterns across the samplings of behaviors are in line with our earlier findings of relative stability in altruistic behavior. Intensity, aggression, escalation, duration, and emotional elements are more distinguishing of stable individual differences than are overall frequecies. Boys exhibit somewhat greater stability than girls. Although boys are more likely than girls to be classified as high in interpersonal aggression, the majority of boys and girls are very similar in interpersonal aggression. Boys are more aggressive than girls toward property. Boys with a high intensity of aggression appear to be easily emotionally aroused generally. They are more emotionally expressive in both positive- and negative-arousing situations. They are also more likely to react altruistically to others' distress. The aggressive patterns of girls are not as consistent; they appear more likely than boys to make reparations for their acts of aggression.

As part of the experimental procedures in which children are observed with peers, the affective environment is altered by introducing two adults who, in the background, interact with each other either in a very friendly manner or in an angry quarrel. Exposure to anger brought out a predominant affect of distress in the children, and temporarily inhibited aggression between peers. After exposure, however, time spent in peer aggression increased significantly. The increased physical aggression was not imitative. The arousal by the exposure appeared to reduce the child's capacity to regulate aggressive impulses. The effect of ambient anger on the easily aroused, high-aggressive boys is especailly marked. These boys appear to be at risk in a number of ways . Many of them show insecure attachments to mother, which suggests that they face a less than warm emotional climate in the home. Such negative affective environments coupled

with high emotional responsivity could provide the conditions for spiraling aggression by child and parent. These data mark an early critical vulnerability and an important time and kind of needed intervention.

Parental Psychopathology and Child Development

By far the greatest research commitment in the Laboratory is studies of child development in relation to rearing experiences provided by seriously disturbed parents. Three projects address the problem directly: a study of families in which parents are physically abusive of their children (Drs. Penelope Trickett and Elizabeth Susman); a study of manic-depressive parents and their children (beginning at age 1 and followed into childhood) (Drs. Carolyn Waxler, Leon Cytryn, Donald McKnew, Marian Yarrow, Mark Cummings, and Ronald Iannotti); a study of depressed mothers (Drs. Marian Yarrow, Carolyn Waxler, Leon Kuczynski, Leon Cytryn, and Donald McKnew). All studies have normal comparison groups.

One study focuses on the emotional development of the physically abused child and the relationship between this development and aspects of the rearing environment of the child. While clinical evidence shows that abused children are at risk for a wide range of physical and emotional problems, few controlled empirical studies exist, and there is no research which relates specific aspects of the enduring environment of the abused child to the child's development. Through referals from Protective Services, families of middle and lower middle class background are studied. Mother, father, and child are observed under naturalistic and experimental conditions. Parents' reports are also obtained. Initial analyses indicate that abusive families do not fall into homogeneous classifications on many rearing dimensions. There is a tendency for abuse by fathers to be associated with philosophies of rearing that advocate stern, harsh discipline. When mothers are the abusers, this behavior is more likely to be linked with high stress levels and psychopathology. Feelings of depression among the mothers are significantly more frequent in the abusing than in the normal mothers. Abused children have significantly more behavior problems of both externalizing and internalizing kinds. Girls tend toward internalizing and boys toward externalizing behavior problems.

Two projects are concerned with diagnosed parental depression. The expressions of parents' illness in their rearing behavior and in the behavior of children of these parents are investigated. The first project focuses on children of manic-depressive parents compared with matched normal families. The recurrence of manic-depressive illness across generations is well documented, evidence for a genetic predisposition to the disorder. The affective environments in which children of manic-depressive parents are reared may also play a significant role in the transmission process. The high levels of stress and distress that characterize manic-depressive families, the unpredictability of parental affect and behavior, parental difficulties in establishing good interpersonal relationships, and possible atypical child-rearing practices in these families are affective and behavioral events that may deviate from those experienced in normal family environments. Study of the families began when the child was one year of age; they have been

followed for two years. Experimental procedures (such as the attachment paradigm, altered ambient environment, play settings with peers), observations in the home, and interview data have been utilized. Findings include the following: In bipolar families compared with the normal control families, mothers are more disorganized, are more inconsistent in caregiving, have more feelings of anger, fear, and sadness, are more likely to have negative feelings about the child, and are less likely to be open in their expression of affect toward the child. The children from manic-depressive families have significantly more frequent and severe behavior problems, expressed in a wide range of symptoms. Their attachment patterns are disturbed, as are their social-cognitive competencies. They are hypersensitive to distress states (particularly anger and aggression) in others, but are less altruistic than children from normal families. They do not show more aggression than children from normal families but their aggression is more undirected and not leading to conflict resolution. Although positive coping skills are present, a surprisingly large number of symptoms and problems surfaced in these families. There are implications for interventions.

Growing out of this study is a much more extensive investigation of mothers diagnosed (SADS-L) as depressed (bipolar, major and minor, unipolar, and intermittent) and normal mothers and their two-year old and five- to eight-year old children. For this study, a method was developed whereby rearing behaviors are directly observed. Mothers and children are observed with each other under conditions that maximize the advantages of naturalistic observation and realize as well some of the control of laboratory conditions. The families are seen over a series of half-days, in a laboratory setting that is an informal, home-like apartment. Conditions and facilities allow and encourage usual daily routines, demands, and interactions. Each session has, however, an underlying controlled structure in which certain standard events occur. Psychiatric assessments are made of each child, independent of the observational data and blind as to mother's diagnosis. Additional test and interview data are obtained. Forty families have been seen to date. Each family will be seen at intervals over the next two to three years. Biological assessments are projected in the follow-up measurements.

It should be noted that the environmental contribution to the transmission of patterned behavior in the children of parents with affective illness has had little direct and detailed investigation. Likewise the early behavior of children of depressed parents and its transformation in the course of development has not been systematically investigated. The rearing paradigm used in the present study is one that is generally applicable and informative concerning rearing-child behavior relations. A detailed study of aspects of parental regulation of child behavior, by Dr. Kuczynski, is a design within the larger design which will permit us to examine this aspect of parent behavior.

Very preliminary analyses, comparing normal and depressed families include the following observations: 87% of the 5- to 8-year-old children of the lower economic class depressed mothers, 40% of the children of the middle class depressed mothers, and 33% of the children of the middle class normal mothers received diagnoses. In the lower economic group, diagnoses were of a depressive disorder in 71% of the cases; in the middle-class group diagnoses were of a depressive disorder in 29% of the cases. Depressive items on the Achenbach Scale for the children of the middle class depressives reflected self-criticism, guilt, and concerns with perfection. For the children of the lower class, the items revealed themes of ambivalence, dependency, and despair. There is a significant relation between aggression and depression in the children of the depressed mothers. These findings must be regarded as tentative. It is still too early in the research to undertake major analyses of the data.

Scientific and Professional Contributions

This year, as in former years, investigators in the Laboratory have had siginificant influence upon the discipline of Developmental Psychology and upon related disciplines through the research problem areas that they have opened for study, and in the methodological advances they have been made in the study of social and affective processes in child development. The many invitations to give lectures and to participate in conferences at universities and at scientific meetings in the United States, Canada, and Europe reflect recognition of Laboratory contributions. Areas of expertise include developmental psychopathology, emotional development, child health and behavior interrelations, adolescent problems, and observational research methodology.

Scientists in the Laboratory continue to have a broad impact on research relating to children through editorial and research review activities. Staff members serve on the editorial boards or as consultant editors for major journals in the discipline: Child Development, Developmental Review, Journal of Applied Developmental Pscyhology, Journal of Educational Psychology, Journal of Consulting and Clinical Psychology, Journal of Autism and Developmental Disorders, Developmental Psychology, Infant Behavior and Development, Merrill-Palmer Quarterly, Child Care Quarterly, and Prevention and Human Services. The staff members are active in the major scientific and professional societies of the disciplines, serving on committees and participating in programs of the American Psychological Association, the Society for Research in Child Development, the American Psychiatric Association, the International Society for the Study of Behavioral Development, and others. Members are frequently called upon for research review functions--by the societies just mentioned as well as by the National Science Foundation, review boards of other institutes at NIH, and the Canada Council.

Scientists in the Laboratory have contributed to the official "Continuing Education" programs for psychologists and psychiatrists by giving invited lectures. Also, this year Dr. Yarrow met with a committee of scholars from the Peoples' Republic of China, who have the responsibility for planning the China Child Development Center to be established in Beijung.

The research of the Laboratory has been widely reported in the media -- in major newspapers (the New York Times), professional news services (Clinical Psychiatric News, Pediatric News), and magazines (Parents' Magazine, Eltern [West Germany], and Psychology Today).

Investigators in the Laboratory are engaged in collaborative research with scientists in other NIMH laboratories, other institutes (NCI, NIAMDD, NICHD), Office of the Director of the Clinical Center, children's hospitals across the country, social agencies, the University of Southern California, and the Medical Center of the University of Colorado. In addition, members serve on committees within NIMH, ADAMHA, and NIH. Dr. Yarrow is a member of the Board of Scientific Counselors of the National Institute of Aging.

Scientists in the Laboratory have been the recipients of two awards this year. The Society for Research in Child Development and the Child Development Foundation made a Conference Award to Drs. Carolyn Waxler, Mark Cummings, and Marian Yarrow. With the collaboration of NIMH, a conference on "The development of aggression and altruism: biological and environmental origins" was held in Bethesda. It brought together 20 scholars from universities in the United States and Canada. A major aim was to foster an interdisciplinary approach to the study of aggression and altruism. A book publication of papers presented at the conference is being prepared. The Outstanding Achievement Award of the University of Minnesota was given to Dr. Marian Yarrow, "in recognition of former students who have attained distinction and honor in their respective fields."

Staffing

A comment about staffing and research facilities: The research of the Laboratory has suffered seriously over the past year as a result of staffing limitations and reduction-in-force procedures. Projects have been disrupted in midstream with changes in research staff who were in key roles of data collection and analysis. One quarter of our support staff who were highly and specifically trained to carry out specialized research procedures, nursing functions, and data analysis have been replaced by persons, through reduction-in-force procedures, who are sometimes without any (or relevant) research training or experience and who must now be trained, over many months, to fill research functions. The loss in time and expertise is costly.

Fortunately the Laboratory has attracted many graduate and undergraduate students from local and distant universities. These students have contributed greatly to our research operations by carrying out university approved "practicums." Students who are Normal Volunteers at the Clinical Center have also assisted in research-support functions. Although they are generally here for only a few months, these students have given splendid help in data collection and analysis.

In several instances, help from volunteer sources has been especially advanced and consistent over the year: Dr. Linda Stern, who completed her doctoral thesis on children of depressed mothers, worked collaboratively on our project. Dr. Ruth Wylie, professor of psychology at Goucher College, has been a most valuable contributor in the same study.

This year Dr. Howard Moss retired. He has been a long time member of the Laboratory and is a productive and internationally recognized developmental psychologist. He is known especially for his research on infant development in relation to maternal care, and, more recently, for his research on the effects of cranial irradiation, used in the treatment of childhood leukemia, upon cognitive functioning. Fortunately, he has returned to the Laboratory on a part time basis as a Guest Worker.

Research Facilities

The physical research facilities of the Laboratory (a large, attractive house on the NIH campus) continue to be especially well suited to behavioral studies of children and families. We are now able to relieve crowded space by carrying out a variety of research procedures (such as interviewing and bio-medical assessments) in the Ambulatory Care facilities of the Clinical Center.

The Future

Most of the projects described are programatic and relatively long term. We would anticipate their continuation, and with the accumulation of research participants and additional data that comes with time, we will be in an ever better position to answer some of the questions we are investigating, and to contribute to our understanding of child development. In our studies of parent and child psychopathology, we anticipate the incorporation of biological assessments and therewith, additional research problem formulations. Progress in these directions will depend on working out collaborative research endeavors with appropriate biological laboratories in NIMH or NIH. Continued progress depends on maintaining and recruiting staff of high caliber who represent the disciplinary diversity that is required in carrying out the kinds of studies in which we are engaged.

Annual Report of the Laboratory of Neuropsychology

National Institute of Mental Health

Mortimer Mishkin, Ph.D., Acting Chief

October 1, 1981 to September 30, 1982

Two cortical visual pathways

Converging evidence from our earlier neurobehavioral, physiological, and anatomical studies indicate that the striate cortex in the monkey is the source of two multisynaptic corticocortical pathways. One courses ventrally interconnecting the striate, prestriate, and inferior temporal areas and is crucial for the visual identification of objects; links between this occipitotemporal pathway and limbic structures in the temporal lobe as well as ventral portions of the frontal lobe appear to make possible the cognitive association of visual objects with other events, such as emotions and motor acts. The other visual pathway runs dorsally, interconnecting the striate, prestriate, and inferior parietal areas and is critical instead for the visual location of objects; links between this occipitoparietal pathway and both dorsal limbic and dorsal frontal cortex enable the cognitive construction of spatial maps, as well as the visual guidance of motor acts that were initially triggered by activity in the ventral pathway. In contrast to the occipitotemporal pathway, which remains modality specific throughout its course, the later stations in the occipitoparietal pathway receive convergent input from other modalities and so constitute polysensory areas.

We have now demonstrated the entire cortical visual system at work by use of [^{14}C]2-deoxyglucose method developed at NIMH by Sokoloff and his group in the Laboratory of Cerebral Metabolism. In collaboration with that laboratory we have found by comparing a blinded and a seeing hemisphere in the same monkey that all tissue related to vision can be clearly identified on the basis of differential hemispheric uptake of the 2-deoxyglucose during visual stimulation. The studies were carried out in awake monkeys presented with visual patterns either in a rotating drum or in a discrimination apparatus. In the latter case, the animal performed the discrimination for water reward using the hand opposite the blind hemisphere. In both situations reduced glucose utilization in the blind as compared with the seeing hemisphere was found cortically throughout the entire expanse of striate and prestriate cortex (areas OC, OB, and OA), inferior temporal cortex as far forward as the temporal pole (areas TEO and TE), and inferior prefrontal cortex (area FD_v). Dorsally, the area of reduced LCGU included the posterior part of the inferior parietal lobule (area PG) and prearcuate cortex (area FDA). These results, which are in remarkably close agreement with our neurobehaviorally derived model of the two cortical visual pathways, have allowed us to delineate the exact limits of the entire system, the visual/nonvisual borders having turned out to be sharp and highly consistent among animals. These borders outlined more cortical tissue than is generally recognized as being related to vision, including the fundus and part of the upper bank of the

intraparietal sulcus, the posterior part of the hippocampal gyrus, and the lateral bank of the rhinal sulcus. Also, the borders appeared reliably at zones of architectonic transition, lending new functional validity to cortical architectonics. Finally, tissue related to vision could be traced subcortically into parts of the limbic and striatal systems, findings that are relevant to later sections of this review.

Organization of prestriate cortex

To trace the flow of visual information through the large expanse of visual cortex revealed by the metabolic studies, we have undertaken a series of mapping studies using axonal transport and axonal degeneration techniques. Our goal in these anatomical investigations has been to identify the multiple visual areas within the prestriate cortex, explore their organization, and map their projections forward into both the temporal and parietal lobes. In the course of these studies, we have developed a myeloarchitectural stain that clearly distinguishes among the multiple prestriate areas for the first time.

Our findings thus far confirm the prediction that the striate cortex is the source of two major cortical projection systems. The first system begins with the striate projection to the second visual area, V2, which in turn projects to areas V3 and V4. These three prestriate areas are arranged in adjacent "belts" that nearly surround the striate cortex, and, like striate cortex, each belt contains a complete representation of the visual field. Area V2 corresponds to prestriate area OB, while V3 and V4 are both contained within prestriate area OA, exclusive of its dorsal part. Area V4 in turn projects to both areas TEO and TE in the inferior temporal cortex.

The second major system begins with both striate and V2 projections to visual area MT, which is located in the caudal portion of the superior temporal sulcus, mainly within dorsolateral OA. Area MT in turn projects to four additional areas in the upper superior temporal and intraparietal sulci. Although the total extents of these four areas are not yet completely established, the more anterior one in the intraparietal sulcus clearly falls within area PG. Thus, one major system of projections out of striate cortex is directed ventrally into the temporal lobe, while a second is directed dorsally into the parietal lobe. Furthermore, the divergence between these two systems appears to begin almost immediately after striate cortex, i.e., in its initial projections.

The two multisynaptic projection systems that we have traced constitute part of the anatomical substrate for object vision and spatial vision, respectively. In addition, our anatomical results provide a partial solution to a puzzle that has persisted in the literature for decades, namely, why extensive bilateral removals of prestriate cortex in monkeys have repeatedly failed to yield the expected losses in either object or spatial vision. If prestriate cortex constitutes an essential relay in both a striate-temporal and a striate-parietal pathway, then damage to this relay should yield effects at least as severe as damage to both its target areas. Yet such dramatic effects have not been found. The reason appears to be that no prestriate lesion to date has produced a total visual disconnection of the temporal and

parietal lobes, since all removals have spared varying extents of prestriate tissue that could continue to relay visual information. Comparison with our anatomical maps indicates that the portions of prestriate cortex that have consistently escaped damage are those parts of both the belt areas and the MT-related areas that represent the peripheral visual fields. Thus, just as we and others have found with sparing of peripheral field representations in striate cortex, such sparing in prestriate cortex will protect both object and spatial vision from serious losses.

Although the basic reason that prestriate ablations have failed to yield the anticipated visual deficits has thus been clarified, the problem created by the negative findings is still not completely solved. For, unlike animals with lateral striate lesions, in which the spared peripheral field representations constitute fully half of the total extent of striate cortex, animals with some of the more massive prestriate lesions that have been reported in the literature had spared peripheral field representations that constitute less than one-third of the total prestriate cortex; and even this is undoubtedly an overestimate, since some of the tissue that survived was presumably disconnected from its visual sources or targets by invasion of the white matter in the preoccipital region. Finally, the prestriate tissue that did remain functional was necessarily divided among the many prestriate subdivisions that have now been discovered, all of which need not have represented precisely the same portion of the peripheral visual field. The question remains, therefore, how such prestriate remnants serving peripheral vision can continue to receive, process, and transmit sufficient information to allow the later stations in the two visual pathways to perform their highly complex visual functions at only slightly reduced levels. Only when the details of visual processing through the two cortical pathways are fully understood will this prestriate-lesion paradox be fully resolved. To gain some insights into visual processing we have undertaken a number of electrophysiological studies.

Prestriate cortex and the analysis of motion and figure-ground relationships

Now that we know the location, topography, and connections of many of the prestriate subdivisions we can compare their neuronal properties and trace the transformation of visual information through them. So far, we have recorded from two prestriate areas, V4 and MT, and have found striking evidence for functional specialization in both. In area MT, it has been known that neurons are not sensitive to the form or color of a stimulus but are highly sensitive to the direction of stimulus motion. We have discovered an elaborate columnar system for direction of motion in MT, comparable to the columnar system for orientation discovered by Hubel and Wiesel in striate cortex. Within a vertical column in MT, all cells respond to the same direction of motion. Moving across the columns, the prefered direction of motion changes systematically, resulting in a clock-like representation of direction. The sensitivity of MT neurons to where a stimulus is moving, rather than to what the stimulus is, is consistent with the strong anatomical connections of MT with areas of the posterior parietal cortex, which is necessary for processing the spatial relationships among objects. By contrast, in area V4, neurons are not sensitive to motion but are highly sensitive to object contours and color. In addition to sensitivity to the length, width, orientation, color,

and contrast of object contours, many V4 neurons respond to a stimulus only if it stands out from its background on the basis of a difference in color or contrast. Thus, V4 neurons may play a role in separating figure from ground, a fundamental task in visual perception. Like the properties of MT, those of V4 are also consistent with its anatomical connections, since V4 provides the crucial link in the relay of information along the ventral pathway for object vision into the inferior temporal cortex.

Inferior temporal cortex and the analysis of shape

The discrimination of complex visual stimuli normally depends on a variety of features, such as brightness, size, texture, and color, but probably the most salient visual feature is shape. In previous studies of the properties of inferior temporal neurons, we found that most neurons were in fact highly sensitive to the shapes of objects. Since very few of these neurons are sensitive to the local features of objects, such as the location and orientation of single edges, they presumably code some global measure of shape. The problem is to identify that measure.

It is well known from the computer pattern-recognition literature that a particularly potent global measure of shape is the Fourier descriptor. The Fourier descriptors are a basic set of shape functions that can be used to generate any shape; likewise, any object can be decomposed into a unique set of these shape descriptors. The descriptors thus provide a powerful method for classifying shapes. Is it possible that inferior temporal neurons use such a method?

To answer this question, we have been studying responses of inferior temporal neurons to a set of Fourier descriptors. So far, over half the neurons tested have been sharply tuned to the set, that is, each of the tuned neurons responds best to a particular descriptor in the set. Furthermore, most of the tuned neurons exhibit size constancy in that they respond best to a particular descriptor regardless of size. These results strongly support a Fourier descriptor-like model of shape analysis. We are continuing to investigate other models of shape analysis, however, especially-spatial frequency decomposition, to determine if the Fourier descriptor model is in fact the best explanation for our results.

Whereas the foregoing single-unit studies are being carried out on lightly anesthetized and immobilized preparations, others are being performed on the awake, behaving monkey while the monkey's fixation is closely monitored by a magnetic search-coil system. In our initial experiments with this technique, we found that active fixation of a tiny spot exerted an unexpectedly strong suppressive effect on neuronal responses to visual probe stimuli. If the fixation spot was turned off briefly while eye position was maintained, the response of the neuron to the probe stimuli increased dramatically. The improvement of neuronal responses to the probe stimuli when the fixation spot was absent could not have been due to a shift in attention to the spatial location of the probe stimuli, for when such a shift in spatial attention was explicitly required (by requiring the animal to respond to a slight dimming of the probe rather than of the fixation spot), the neuronal responses to the

probe stimuli were weakened even more. In short, both the physical presence of the fixation spot and spatial attention to the probe were suppressive influences. This suppressive effect of attention to the probe stimuli, however, was obtained on tasks in which the shape of the probe stimuli was irrelevant for correct performance. In subsequent studies we made the shape relevant, by requiring the animal to discriminate between differently shaped probes, and under these conditions the strength of the neuronal responses to the probe stimuli was dramatically enhanced. Besides providing additional evidence that inferior temporal neurons are engaged in processing stimulus shape, these experiments show for the first time that the responses of inferior temporal neurons are markedly altered depending on which stimulus feature, location or shape, the animal is attending to. The results suggest the interesting possibility that the dorsal cortical visual pathway, specialized for spatial vision, is responsible for the suppressive influence that attention to spatial location exerts on the activity of inferior temporal neurons. Study of the functional interaction between the two cortical visual pathways at the neuronal level should provide valuable new insights into the mechanisms underlying selective visual attention.

The anatomical experiments described earlier also bear upon this issue of selective attention. Materials from the anatomical experiments were used to investigate not only the cortical projections from striate cortex but also its subcortical projections to the pulvinar nucleus in the thalamus. In the corticocortical studies we had found that striate efferents to the prestriate area V2 (or OB) are visuotopically organized, and we had prior evidence of a similar topographic arrangement of pulvinar projections to V2. In the study on striate efferents to the pulvinar, we found that once again there was a precise visuotopic organization, indicating that there are two sources of striate input to V2 in perfect register: one, direct, i.e. corticocortical; and the other, indirect, via the pulvinar. The latter system of inputs to V2 provides a possible mechanism by which signals (instructions, sets, etc.) from outside the visual system operating through the pulvinar could acquire visual field specification and thereby direct the animal's attention to a particularly part of the visual field. Future anatomical studies will investigate these possible sources of nonvisual input to the pulvinar.

Inferior temporal cortex and stimulus equivalence

According to our earlier unit-recording studies, nearly two-thirds of inferior temporal neurons receive information from both hemispheres (i.e., from both the left and right visual hemifields) via the forebrain commissures. We have therefore proposed that inferior temporal cortex is important for stimulus equivalence across the visual fields. This is the phenomenon in which a stimulus is recognized as the same regardless of its retinal position, even if the different retinal loci project to different hemispheres. To test our hypothesis, we prepared monkeys with bilateral inferior temporal ablations combined with section of the optic chiasm and trained them first with one eye and then with the other eye on a series of visual pattern discriminations. Unlike control monkeys (with either inferior temporal lesions alone or chiasm-section alone), who can perform immediately with the second eye any

discrimination that they have learned with the first, the experimental monkeys had to learn each discrimination anew with the second eye. These results provide strong evidence that stimulus equivalence across the visual hemifields depends on the convergence of their projections onto single inferior temporal neurons. We are currently testing a further prediction from this hypothesis, namely, that inferior temporal lesions will prevent transfer of a visual pattern discrimination from one locus to another within one hemifield. The method involves training monkeys whose maintained fixation is being monitored as described earlier to discriminate a pair of patterns in one quadrant of a visual hemifield and than test them for transfer to the other quadrant. Transfer should occur, as before, only if inferior temporal neurons are present to mediate the spatial convergence of visual information. In a related electrophysiological experiment (see preceding section) we have determined that, in fixating monkeys performing a discrimination parafoveally, single inferior temporal neurons are indeed activated by their adequate stimuli irrespective of stimulus position in their large receptive fields.

Not only inferior temporal but all areas within the cortical visual pathway are known to be reciprocally connected through the forebrain commissures. In particular, the representation of the vertical meridian at the OC-OB border as well as selected parts of area OA receive commissural inputs via the splenium of the corpus callosum, while extensive portions of posterior temporal cortex, area TEO, like the more rostral temporal cortex, area TE, receive their contralateral input via the splenium and anterior commissure. Since the transfer of visual information between the hemispheres is critically dependent on these reciprocal connections, we have attempted to localize and to quantify their contribution to vision by application of the 2-deoxyglucose method. In this experiment, rates of local cerebral glucose utilization (LCGU) were measured throughout the cortical visual system in two different surgical preparations: unilateral optic tract section combined with forebrain commissurotomy, and unilateral optic tract section alone. The commissural contributions to vision were inferred from differences in LCGU between the deprived hemispheres of the two groups.

Results indicated that there were no differences between operated groups in the visually deprived hemisphere for areas OC through TEO, where LCGU averaged 50% of that in the intact hemisphere. A difference attributable to visual input via the intact commissures was found in TE, however, where LCGU in animals with combined tract section and commissurotomy remained at 60% of that in the intact hemisphere, whereas, in animals with tract section only, LCGU reached 80% and 90% of the values in the intact hemisphere for posterior and anterior TE, respectively.

These results have presented us with a paradox. On the one hand, our metabolic data on area TE are in good accord with existing anatomical data, which indicate that area TE receives interhemispheric projections through both the splenium and the anterior commissure. On the other hand, the same metabolic data give no indication of the equally heavy commissural projections to the more posterior visual areas - specifically, the OC-OB border, large parts of OA, and throughout TEO. One possible explanation for this paradox is that, unlike the commissural input to area TE, which clearly can support visual function by itself, the commissural input to the more posterior zone

may be effective only against a background of activity provided by an intact retino-geniculo-cortical pathway. To test this hypothesis, we have prepared a series of monkeys in which a "blind" right hemisphere was produced by midline section of the optic chiasm combined with occlusion of the right eye, rather than by right optic tract section. Through a comparison of LCGU in the right hemispheres of these animals and of those done previously with right optic tract section, the functional effectiveness of commissural input with and without spontaneous retinal input may be evaluated. Greater metabolic activity in the former case would indicate that the commissural fibers to the prestriate-posterior temporal zone do require a minimum level of background activity from the intact retina in order to make a functional contribution to vision. Preliminary qualitative analysis, however, has failed to reveal any reliable difference between animals with a tract cut and animals with a chiasm cut at either the OC-OB border or within areas OB, OA, or TEO. In short, the commissural contribution to the prestriate-posterior temporal zone, unlike that to area TE, is not reflected metabolically. Unit-recording studies are now being planned to investigate this still unexplained functional difference within the commissural system for vision.

Inferior temporal cortex and object recognition

The spatial convergence upon single cells in area TE provides a mechanism that is essential for discrimination learning under normal viewing conditions. It insures that the same central visual cells will be activated from one exposure of the stimulus to the next despite the fluctuations in fixation, distance, direction, and angle of regard that lead to stimulation of different populations of striate and prestriate cells on different presentations. But spatial convergence alone is insufficient to insure reactivation of the same central visual cells unless it is associated with a mechanism for temporal convergence. That is, a given stimulus must excite the same visual neurons on a second occasion as on the first if there is to be any summation of the effects of experience or training. We now have evidence that the inferior temporal cortex does indeed contain a mechanism for temporal convergence, as indicated by the following experiments on visual recognition.

Trained monkeys that are shown an object once will demonstrate that they recognize it as familiar several minutes later by avoiding it in favor of another object that is completely novel. Thus, somewhere in the visual system the single presentation of a complex stimulus leaves a trace against which a subsequent stimulus presentation can be matched. If it does match, i.e., if the original neural trace is reactivated, there is immediate recognition of familiarity, and the behavioral consequence described above ensues. The area in which the neural trace is first established turns out to be area TE, since removals here but not elsewhere in the visual system abolish the animal's ability to recognize an object that it has seen once just a few seconds before. Apparently, area TE contains the traces laid down by previous viewing, and these serve as coded and stored representations against which incoming stimuli are constantly being compared. In the process, old traces may either decay, be renewed, or even refined, and new traces are added to the store. An experiment is now being designed to study these postulated visual traces at the single-unit level. The question to be addressed is whether or not a neuron that at first responds equally to two similar stimuli can be

induced to respond differently to them by training the animal to attend to their physical differences.

A limbo-thalamic circuit and object recognition

In the process of investigating the role of other temporal-lobe structures in object recognition, we obtained a result that is particularly exciting because it may help to solve a long-standing puzzle concerning the neuropathology underlying the syndrome of global amnesia in man. This syndrome, which is characterized by a profound inability to remember new experiences, has been attributed in the clinical literature to destruction of the hippocampus. Yet, attempts to duplicate this syndrome in animals by removal of the hippocampus have largely failed. What we have found in our studies is that if damage to the hippocampus is combined with damage to the amygdala then a profound recognition loss ensues, a loss that is even greater than that produced by lesions of inferior temporal cortex. The discovery has not only opened up a new possibility for resolving the discrepancy between clinical and animal findings but has also led to new insights into the neural mechanisms of memory.

According to our present model, object recognition depends on a reciprocal cortico-limbo-thalamic pathway, activation of which leads to the storage in cortex of the trace or representation of the stimulus that gave rise to the activation. In the case of vision, as already indicated, the cortical tissue in which the storage is presumed to take place is area TE. However, as will be described in a later section of this report, other sensory modalities appear to be organized neurally along lines similar to those of the visual modality, and each is presumed to be represented by a highest-order processing station located in the anterior temporo-insular region with functions analogous to those postulated for area TE. We know that each of these highest-order sensory processing stations projects both to the amygdala and, via relays in the perirhinal and entorhinal cortex, to the hippocampus. Furthermore, we have shown that, in vision, one-trial object recognition is severely impaired not only by area TE lesions, or by combined amygdalo-hippocampal lesions, but also by disconnection of area TE from its two limbic targets in the temporal lobe.

In our most recent studies, we have found that the limbo-thalamic portion of the pathway actually consists of two relatively independent limbo-thalamic segments, one from the amygdala through the amygdalofugal pathways (AFP) to the magnocellular portion of n. medialis dorsalis (MDmc), and the other from the hippocampus through the fornix (Fx) to the anterior thalamic nuclei (Ant. N). The evidence is based on comparison of the effects of separate and combined AFP and Fx transections, as well as of separate and combined MDmc and Ant. N ablations. In both cases, the combined lesions yielded significantly greater recognition losses than did the separate lesions. Because of their newly discovered functional significance, the two limbo-thalamic projection systems are now being mapped in detail with axonal transport techniques. The initial results show that the amygdaloid projections arise throughout the complex, though most heavily from the basomedial nucleus, sweep through the substantia innominata, and then travel in the inferior thalamic peduncle to

enter the head of the thalamus before passing caudally to terminate in MDmc and n. reuniens; allocortical areas adjacent to the amygdala also contribute significantly to this projection. Hippocampal projections arise predominantly in the subiculum and terminate most heavily in nuclei anterior medialis, anterior ventralis, and lateralis dorsalis, with lighter projections to nuclei reuniens, centralis latocellularis, rotundis, and paraventricularis; all of these projections course through the medial part of the fornix, though n. lateralis dorsalis also receives a nonfornical input which runs through the medial pulvinar. New studies are being undertaken to look at the effects on recognition memory of damage to other diencephalic targets of the limbic system, such as the mamillary bodies, as well as to the prefrontal cortical targets of MDmc and Ant. N.

Amygdala, hippocampus, and associative memory

Once the trace or representation of a stimulus has been stored in the highest-order processing station for any given modality, that stored trace can enter into association with the stored traces of other stimuli and other events, thereby providing the stimulus with associative meaning. As has been indicated, the amygdala and hippocampus, as well as their separate efferent pathways and separate thalamic targets, make approximately equal contributions to recognition memory; according to our neural model, this reflects their roughly equal contribution to the cortical storage of stimulus traces. In the case of associative memory, however, our results indicate that the amygdala and hippocampus make very different contributions.

In one experiment, monkeys were trained preoperatively on a visual recognition task and, separately, on a tactual recognition task, with the same set of objects comprising the stimuli for both modalities. One group of monkeys then received amygdalectomies and the other, hippocampectomies, after which both were retrained on the intramodal memory tasks to a high level of performance. When tested later for their ability to perform the recognition task across modalities, i.e. to choose between two objects visually after one had been presented as a tactile sample, the hippocampectomized monkeys continued to perform at a high level, but the amygdalectomized monkeys fell to chance performance.

Nearly the opposite results were obtained in a second study that tested the ability of monkeys to remember the spatial location of visual objects. In this task, the animal was required to remember on the test trial where on a three-well tray each of two different objects had been presented on the acquisition trial. In this case, monkeys given amygdalectomy were able to regain the level of performance they had achieved preoperatively, whereas those given hippocampectomy failed to rise above chance.

The results of these two complementary experiments indicate that although both the amygdala and hippocampus are important for associative memory, their roles are totally different. Many further analyses along the lines of these two experiments are needed, however, before the selective associative memory functions of the amygdala and hippocampus can be precisely identified. For example, the association of an object with an affective state, such as fear,

pleasure, etc. appears to depend much more heavily on the amygdala than on the hippocampus. New support for this view is being obtained in an experiment showing that one-trial object-reward association is impaired far more by amygdaloid than by hippocampal lesions. By contrast, because of the contribution to spatial memory that is made by the hippocampus, the association of objects with spatially directed motor acts could depend more heavily on the hippocampus than on the amygdala. Studies to examine this possibility are being planned.

A cortico-limbic pathway in somesthesis

The work described above elucidating a cortico-limbic system for visual perception and memory has led to the search for analogous systems in other modalities. So far, analogous anatomical pathways have been found for both taste and audition, but because of early difficulties in establishing such a pathway in somesthesis, particular attention has been paid to this modality. Also, anatomical studies on the somatosensory system have been supplemented by neurobehavioral and, more recently, electrophysiological studies, in order to permit functional comparisons with the cortico-limbic system in vision.

Previous anatomical and electrophysiological studies had indicated that both the superior parietal lobule (area PE) and the second somatosensory area (SII) were possible relays in a processing pathway connecting the primary somatosensory area (SI) with the limbic system. To evaluate these possibilities behaviorally, monkeys with lesions of either area PE or SII were compared with normal controls in the ability to differentiate objects on the basis of texture, shape, and size. The results indicated that SII, but not area PE, is necessary for such tactual discrimination. This finding led, in turn, to the search for a pathway linking SII with the limbic system. Although our earlier experiments yielded ambiguous results, recent studies employing both anterograde and retrograde axonal transport techniques have provided positive evidence for such a pathway.

Injections of cell markers were made into the hand representation of physiologically identified cortical fields lying in or near the lateral sulcus of the monkey. These regions include SII, area PF, the retroinsular cortex (Ri), and the granular and dysgranular insular fields (Ig and Id). The results revealed reciprocal connections between SII and Ri, SII and area PF, SII and Ig and Id, and Ri and Ig. In addition we have confirmed the previously reported reciprocal connections of SI and SII, and demonstrated reciprocal projections between area PE and both Ri and area PF.

The laminar pattern of termination of these projections suggests the sequential order in which the somatosensory fields process information. In the visual system, forward corticocortical projections (i.e. outward from striate cortex) are characterized by a heavy input to layer IV, while backward projections (i.e. towards the striate cortex) completely avoid layer IV and instead have a characteristically heavy input to layer I. By analogy with the visual system, an analysis of the laminar pattern of termination in our material indicates that the forward sequence of projections is: SI to SII and area PE, area PE to Ri and PF, PF and Ri to SII, and SII and Ri to Ig; SII

also projects to Id. Finally, we have confirmed previously reported projections from Ig and Id directly to the amygdala and indirectly to the hippocampus via the rhinal cortex.

These data demonstrate that a pathway is available for relay of somatosensory information from the SI and SII to the limbic structures of the temporal lobe. Although we have previously demonstrated that combined removal of the amygdala and hippocampus markedly impairs tactual memory, we cannot yet say that the insular cortex is the critical link in this pathway. Experiments examining the effects of insular removals on tactual memory are in progress. In addition, since virtually nothing is known about the physiology of the insular neurons that receive somatosensory input, we are currently examing their response properties in awake monkeys.

Nonlimbic structures and habit formation

On all of the memory tasks that have been described, the deficits are especially severe when removals of the amygdala and hippocampus are combined. Yet, even the combined limbic lesion does not affect all forms of learning and retention. For example, despite their rapid forgetting in one-trial object recognition, animals with the combined limbic lesions have no difficulty learning object discriminations, at least in the standard situation where trials are repeated 3-4 times per minute. In an attempt to resolve this discrepancy between rapid forgetting and successful learning, we tested whether object discrimination learning would be prevented in animals with limbic lesions if intertrial intervals exceeded the putative memory span. Surprisingly, animals with the combined amygdalo-hippocampal lesions learned to discriminate a long list of object pairs even though the list was presented only once every 24 hours. Thus, although the operated animals have an extremely short memory span, they can retain and accumulate information gained from single discrimination learning trials separated by 24-hour intervals. This paradoxical success in the presence of severe memory loss implies the existence of an important retention mechanism outside the limbic structures of the temporal lobe.

We have since performed additional experiments to characterize further the essential difference in function between the limbic and nonlimbic retention mechanisms. Our results suggest that the limbic system is critical for high levels of retention of object-reward associations after a single acquisition trial with short lists of objects, or after two or three repetitions with long lists of objects but short intertrial intervals. With greater repetition, however, retention of object-reward associations can be mediated in the absence of the amygdala and hippocampus, and the retention appears to be independent of both list length and delay. To distinguish this form of retention from memory, we have labelled it 'habit formation'. Further investigation of this mechanism of habit formation as well as elucidation of its neural substrate have become important goals of our research.

Ontogenetic development of memory and habit formation

On the evidence that in the adult monkey there may be two relatively independent systems for retention of information, we recently initiated a series of studies to assess the development of these two systems in infant monkeys. Results thus far indicate that one-trial recognition, requiring memory of one object at a time for only 10 sec each, is absent in infants younger than fours months of age and does not reach adult levels of proficiency even at one year. This slow ontogenetic development of recognition memory was shown even more strikingly with longer delays and lists. In sharp contrast, when 3-month-old infant monkeys were trained on object discrimination habits, they performed exactly like adult monkeys in both acquisition and retention, even though intertrial intervals lasted 24 hours. These results strongly suggest that the two systems of retention that were found to be relatively independent in the adult monkey are also developmentally dissociable. Indeed, they provide evidence that the mysterious phenomenon of infantile amnesia could be due to the absence of a functional memory system in early childhood. On the basis of this evidence, we have begun to prepare monkeys with neonatal removal of this system (i.e. combined amygdalo-hippocampal removals) in an attempt to see how cognitive, emotional, and social behavior develops in animals whose amnesia persists from infancy through adulthood. This study will help to evaluate two provocative proposals from the clinical literature, namely, (a) that early dysfunction of the limbo-thalamic memory system could be one cause of childhood autism, a syndrome characterized by dramatic social and emotional disturbances not seen in adults with the same neuropathology, and (b) that the reason a pure case of amnesia like the one seen in adults has never been reported in a child is that the clinical picture of an amnesic child, being overlaid with autism, is entirely different from the clnical picture of an amnesic adult.

In tandem with these developmental studies of behavior, we have begun to trace the functional development of the visual system metabolically, by applying the 2-deoxyglucose method to infant monkeys in a manner similar to that described earlier for adults. Thus far, a series of animals with unilateral optic-tract section combined with forebrain commissurotomy have been studied at various ages ranging from two days to six months. Preliminary analysis of the results shows that there are systematic age-related changes both in the absolute level of LCGU within the normal seeing hemisphere and in LCGU differences betwen the normal left and the deprived right hemisphere.

As in adults the normal hemisphere shows a gradient in which the absolute level of glucose utilization is highest in Area OC and is systematically reduced in the more anterior areas of the pathway, being lowest in the anterior portion of Area TE. This gradient was present even in the youngest subject, two days old. The steepness of this gradient, however, shows a consistent and nonoverlapping increase with age.

At all ages the deprived hemisphere shows reduced LCGU relative to the normal hemisphere. Also, at all ages, side-to-side differences are greatest in striate cortex and smallest in the anterior portion of the temporal lobe. However, the quantitative differences between the hemispheres change systematically with the age of the animal. Thus, for each cortical area, the relative difference between the right and left hemisphere is smallest at birth

and approaches the difference seen in adults only at about four months. These preliminary results agree with our behavioral data indicating that the neural capacity for visual recognition is probably not developed until about four months of age.

Summary

Through combined use of behavioral and neurobiological methods, we are beginning to discover some of the general principles along which the primate forebrain is organized to serve memory and other cognitive processes. (1) Each primary projection area in the cortex seems to be the source of two multisynaptic corticocortical pathways. Both pathways are composed of several cortical areas that are arranged hierarchically, one pathway being directed dorsally to the frontal motor system, the other ventrally to the temporal limbic system. Before reaching the motor system, the dorsal pathways from the several modalities converge in polysensory areas, which are critical for sensory attention, spatial perception, and motor guidance. The ventral pathways, by contrast, remain modality specific throughout their course and are important instead for stimulus recognition and stimulus meaning, and ultimately for triggering the motor response. (2) Stimulus recognition depends not only on stimulus processing along the ventral cortical pathway but also on storage of a central representation of that stimulus in the ventral pathway's last station, located in the anterior temporo-insular region. This region projects directly to the amygdala and indirectly to the hippocampus, and these two limbic structures project in turn to the medial thalamus. Storage of the central representation of a stimulus occurs only if this cortico-limbo-thalamic pathway is activated. Damage to this pathway results in recognition failure, a core symptom of amnesia. (3) Once the central representation of a stimulus has been stored, it can enter into association with the stored central representations of other stimuli and other events, thereby providing the stimulus with meaning. Thus, stimulus-affect associations probably depend largely on cortico-amygdalo-hypothalamic interactions. By contrast, stimulus-place associations appear to depend on cortico-hippocampal interaction; in this case the hippocampus may act as the site of converging information from both the ventral 'stimulus recognition' pathway and the dorsal 'spatial perception' pathway, the latter involving projections from the polysensory areas through the cingulate gyrus to the hippocampus. Finally, stimulus-act associations could depend on interaction between the ventral and dorsal pathways within the lateral prefrontal cortex, which interacts in turn with the motor system. (4) Destruction of the limbic portion of the memory system does not affect all forms of learning and retention, however. At least one form, which has been labeled habit formation, remains nearly intact, presumably reflecting the operation of a powerful nonlimbic mechanism for retention of information. On the basis of behavioral developmental studies, this nonlimbic habit system appears to mature considerably earlier than the limbic memory system.

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Annual Report of the
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Allan F. Mirsky, Ph.D., Chief

This is the second full year report of the program of the Laboratory of Psychology and Psychopathology under the direction of the new permanent Chief, Allan F. Mirsky. The program is still in transition at this time: some older lines of investigation have been discontinued or curtailed, others are being modified, augmented, or redirected and some are being maintained essentially in their current form. In addition, however, certain new lines of investigation are being implemented. Some of these new lines involve the introduction of new personnel into the Laboratory, the acquisition of new equipment, and the construction of new laboratory spaces. In other instances, collaborative efforts have been set up involving the joint efforts of existing and newly-added personnel so as to integrate the pre-existing and new research efforts. Since we have still not occupied our permanent laboratory space, and since acquisition of some of our necessary research equipment is still incomplete, the present summary report is in large part a recapitulation of last year's report. However, we have made extensive contacts with organizations and persons both within and without the NIMH with respect to access to patient groups (schizophrenic, epileptic and brain-injured subjects) and we stand poised and ready to implement the human Studies in Development (Section I, A.) referred to below. The precise scope and direction of the animal model studies described in the subsequent section (I, B) is a bit more uncertain at this time because of concern about the extent of resources in the IRP being devoted to infra-human primate research. Nevertheless, since a substantial portion of this work will ultimately be accomplished, the monkey studies are still listed as being in development.

This summary statement will be divided into two major subdivisions: projects which are in development, and projects in being.

I. STUDIES IN DEVELOPMENT:

A. Human clinical studies of attention disorders

1. The taxonomy of attentional disorders
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I. STUDIES IN DEVELOPMENT:

Introduction

The basic new thrust has to do with the creation of a program of research aimed at developing a taxonomy of attentional disorders. We aim to describe in detail, at a number of levels of scientific analysis, the nature of the system within the brain that provides the substrate for attention, the basic organism/environment interaction. The system involves the participation of many elements within the neuraxis, from phylogenetically ancient structures within the mesencephalon and pons through structures in the diencephalon including some regions of hippocampus, through regions of the temporal, parietal, and frontal cortex. The program of research necessary to describe the attentional system and its manifold dysfunctions involves the study of a number of model or paradigmatic clinical conditions. These include patients with prefrontal, temporal, or inferior parietal lesions. Such cases represent perhaps the clearest instances of brain-behavior relation in that they involve the behavioral effects of specific lesions which can be documented anatomically with a reasonable degree of accuracy. In some of these cases, i.e., lesions including the right inferior parietal lobe, the attentional loss is most pronounced and obvious: the patient may neglect the entire left side of space contralateral to his lesion. The mechanisms underlying the loss, however, are not particularly well understood.

The next most difficult level of analysis involves study of patients with epileptic disorders of the generalized or absence variety. During the absence attack, which some of these patients display in relatively pure form (uncomplicated by even minor motor seizure manifestations), there can be total abeyance of consciousness for varying periods of time. Such absence periods are usually accompanied by dramatic changes in the EEG: generalized, bilaterally synchronous slow discharges of the three per second spike and wave (SW) variety. These discharges allow a specific point of entry into the study of the impaired attention or consciousness; experiments can be designed to analyze behavior or

stimulus processing during, preceding, and following SW discharges, so as to facilitate understanding of the nature of the pathological brain processes involved in the absence attack. The electrographic sign (the SW discharge) thus provides in some sense a temporary lesion which can be the subject of neuropsychological study. Although it is less satisfactory as an experimental variable than the existence of a documented loss of tissue, absence epilepsy does at least provide the opportunity to study each patient as his own control. The brain-injured and epileptic cases provide models and paradigms for the experimental analysis of attention loss which may help in the understanding of the symptoms of impaired attention in clinical states in which the neuropsychological nature of the disturbance is more obscure or not well understood. Instances of these include schizophrenia, infantile autism, and the various types of dementia (e.g., Alzheimer's disease, Korsakoff's syndrome, Huntington's disease). For many of the clinical states under study, the nature of the human clinical material ordinarily precludes the use of certain kinds of invasive experimental methods (e.g., deep probes or implanted electrodes, brain biopsies). Questions arise often as to the locus or nature of the lesion process in absence epilepsy, for example, which can best be approached, given current levels of technology, with animal models. Thus, the role of lesions or other perturbations in specific subcortical brain regions in absence epilepsy can be explored in animal models. Monkeys can be trained to perform complex attention-demanding tasks, and experimental modifications of the animal's brain can be performed: lesions, either static or irritative, of various subcortical structures; electrical or direct chemical stimulation; administration of neurotransmitters, hormones, enzymes, or blocking agents. The effects of such experimental agents in trained, behaving animals can lead to more refined hypotheses about the nature of pathophysiological processes; moreover, models for testing the efficacy of new therapies may be provided by this work. Aside from models of pathophysiology, however, the highly advanced and complex brain of the monkey affords an opportunity
for study of the basic phenomena of attentional brain function in primates. A considerable program of work can thus be followed profitably, leading to analysis of neural processes, both at the level of the individual nerve cell and the nerve network, involved in attention. The broad outlines of projects which will be pursued by the LPP are described below in section B.

A. Human clinical studies of attention disorders

1. The taxonomy of attention disorders: A collaborative project

Many of the members of the LPP are involved in a joint project in which a wide variety of attentional, cognitive, autonomic, and electro-encephalographically-derived tests are applied to the same populations of experimental and control subjects. These will include epileptic persons, schizophrenic subjects, brain-lesioned subjects, dementing subjects, and controls. The aim is to develop a profile of functioning for the several groups that will highlight the similarities and differences among them and will lead to a better neuropsychological characterization of their impairment.

One of the behavioral measures to be used in this study is the Generalized Attention Test (GAT) which is being developed at the NIMH by LPP staff. It represents an extension and modification of the Continuous Performance Test of attention (CPT) which was originally refined and perfected at the NIMH

during the period 1954-1961. The CPT has been used extensively by various groups of investigators, particularly in the study of epilepsy, schizophrenia, and metabolic illnesses. The GAT extends the concept of the CPT to involve a variety of cognitive dimensions (such as intraand inter-dimensional shifts), as well as parametric control of other perceptual variables. The GAT is expected to prove useful in exploring the dimensions of attention impairment in the several clinical groups under investigation in this taxonomy study. The GAT is near completion at this time, and it is likely that many subjects will be run during the next reporting period.

2. Brainstem mechanisms in attention impairment

There are a number of lines of evidence leading to the inference that the classical brain stem systems implicated in attention (in the vicinity of the mesencephalic and pontine reticular formation) are damaged or malfunctioning in certain clinical syndromes. These include absence epilepsy, infantile autism, and possibly, some forms of schizophrenia. This project aims to use existing measures of auditory and somatosensory brainstem functioning which were developed for assessment of neurological (and especially demyelinating) disorders to assess the integrity of these structures in the several clinical conditions.

3. Neurobehavioral studies in absence epilepsy

In this project, specific sensory tests are used to assess functioning before, during, and following absence attacks in patients suffering from generalized seizures of the absence type. This work is done in conjunction with computer-assisted analysis of EEG changes prior to the appearance of SW discharge and has led to an appreciation of the changes in the energy (or more precisely the power) spectrum in cortical areas immediately preceding a paroxysmal EEG discharge. The findings already obtained in this project, which was begun at Boston University, may be considered as elements that can be incorporated in the development of a prosthetic device in treatment of some forms of epilepsy. That is, the EEG changes could be monitored and used to activate a device which would forewarn a patient of impending absence attacks. With help, patients might be able to develop strategies which could prevent, arrest, or abort such attacks. While it is true that most persons with absence seizures can be helped with relatively mild forms of anti-convulsant medications, it is not true of approximately 20% of such cases. These persons require either multiple anti-convulsant drug regimens or near-toxic doses of such drugs. Moreover, even low doses of anti-convulsant medications (whether considered to be easy to tolerate or not) may have deleterious side effects on behavior and biological capacities, especially when they are ingested by young, developing persons for periods of years. Excellent patient sources have been identified, and the cooperation of referring units has been secured. Most of the hardware for this project has been secured or is on order and substantial progress is expected during the next year.

B. Specific animal projects

1. Experimental models of generalized seizures of the absence type

Over the course of the last 15 years, staff now in the LPP have been interested in the development of techniques for simulating the generalized seizure patterns, as well as the behavioral accompaniments, of absence epilepsy. The

tradition of this type of model dates back to research by H. H. Jasper and colleagues (e.g. Hunter, Perot, Ingvar, Pollen, Weir) at the Montreal Neurological Institute beginning in the 1940's. These earlier studies depended for the most part on electrical stimulation of various brain regions, mostly in the midline structures of the diencephalon and mesencephalon. This work led to the concept of the "centrencephalic system" as described by Penfield and Jasper and antedated the "reticular formation" by some years. It also influenced the thinking of many brain scientists about the cerebral bases of attention and consciousness. The neural bases of these behavioral states is still obscure; however, we are still impressed by the potentially close relationship between the brain system involved in generalized seizure discharges and that which is responsible for the maintenance of consciousness. Animal models thus remain important investigative tools in this research effort. We have explored the use of electrical, chemical, and metabolic methods of inducing discharges. In our laboratories, we have used in past studies: electrical stimulation of subcortical and cortical structures, administration of chlorambucil and pentylenetetrazol, subcortical implantation of aluminum hydroxide cream, and application of conjugated estrogen symmetrically to forebrain cortical surfaces. Most recently, we have done some experiments with compounds which block metabolism of GABA (i.e., gamma-vinyl GABA) and produce the paradoxical effect of inducing symmetrical and synchronous SW-like activity in the EEG of the monkey.

Such models of generalized seizure activity permit the testing of various hypotheses and provide an experimental tool for study of single unit activity (as an example) under conditions of seizure.

2. Analysis of cellular activity in the attention system within the monkey brain

The concept of an attention system within the brain has been alluded to in various portions of our annual report. One could adduce evidence for the viability of this concept from a number of approaches or lines of evidence: the effects of lesions and other neuropathological conditions in human subjects; experimental lesions or other manipulations of the brain in animal subjects trained on tasks requiring or designed to accentuate attentive behavior; anatomical-physiological considerations. All of these lines of evidence appear to converge in suggesting that there is a system within the brain that is specialized for the attentional needs of the organism. And one way of characterizing it is to study the attributes of individual nerve cells in those brain regions that allegedly form part of the system. We have conducted studies of two such brain regions to date: the meso-ponto-diencephalic portions of the brainstem and the prefrontal cortex, including both lateral and medial portions. Both of these locations contain large numbers of a particular type of cell which appears to have a specialized function with respect to attention. Our method of identifying this cell type has been based upon an experimental test paradigm in which animals are trained to perform in a go-no go visual discrimination task. Attention-related cells are those which fire in both go and no go trials. That is, they fire on trials when the animal has merely to attend to a stimulus display and performs no obvious motor response except holding a paw on a response key (no go trials); they fire as well on trials on which a brisk lift and hit response to obtain a fruit juice reward is necessary. The cellular response may be identical on both trial types and may in addition anticipate the onset of the cue stimulus by several hundred milliseconds. This type of cell we have labeled as type II and, as noted

above, is found distributed throughout certain regions of the brain stem and prefrontal cortex. Further studies will be aimed at providing a systematic functional analysis of this cell type, searching for other loci in the brain where they may be found, characterizing the relation among the several loci, and beginning pharmacological studies of these cells. As will be documented more fully in the project description Z01 MH 00506-02 below, a gradient of type II through possibly transitional cells through type I cells has been described in certain regions of the monkey frontal lobe. This work suggests that there may exist in the premotor area of the monkey a transitional cell type between the "attention" cell and the motor execution cell. This cell type may serve some bridging or connecting function between the attentional and motor execution systems.

3. Brain lesion and state change effects on visual attention

This series of studies was begun under the auspices of the Laboratory of Neuropsychology and continues temporarily under their aegis until our animal space is prepared. There is collaboration with LCM and SMRC in some parts of this work. One of the goals has been to specify the role of non-visual regions of the primate cerebrum in visual attention, and this work has used several techniques including lesions, stimulation, and electrographic recording of brain potentials under various lesion and non-lesion conditions. Other studies have been concerned with the recording and analysis of visually-evoked potentials under a variety of perceptual conditions, and with drug or sleep state effects on the mechanisms of visual attention.

II. PROJECTS IN BEING:

A. Psychobiology of cognition

The aim of this research has been to relate psychological and biological determinants of various components of cognition that are involved in the acquisition, processing, retention, consolidation, and retrieval of information or experience.

Experiments have been designed to characterize and contrast cognitive failures in patients with disturbances of mood, children with various forms of impairment in learning, and populations of neuropsychiatric patients including those with Huntington's disease, Korsakoff's psychosis, and Alzheimer's disease. How are specific forms of central nervous system changes related to discrete alterations in cognitive processes? Components of information processing in unimpaired individuals are also studied. A third type of study of both unimpaired individuals and patient groups uses various types of drug manipulation to alter specific aspects of cognitive processes. How do drug treatments that alter the activity of discrete aggregates of neurons relate to qualitatively different changes in cognitive processes?

1. Studies of depression

We can now define with some precision the kinds of cognitive processes that are impaired as well as those that are left relatively intact in patients with disturbances in mood (depression). Depressed patients are impaired in aspects of learning, memory, and retrieval. In part, this is a function of the intensity of their depression; moreover, the retrieval of experience is in part

mood state-dependent. However, some aspects of information processing are particularly likely to be interrupted. Using models that distinguish between automatic versus effortful (or passive versus active) processing in cognition, we have noted that depressed patients may be profoundly impaired when cognitive processes require active or effortful as opposed to passive or automatic kinds of operations. For the latter, the depressed patient may be indistinguishable from a normal control. In fact, the extent to which depressed patients are able to manifest effort is highly correlated with the intensity of their depression and with the extent to which they can perform these non-automatic active cognitive operations. Also, the extent to which depressed patients are provided with organization of structure (as opposed to random uses) in information-processing tasks is the extent to which they are relatively indistinguishable from normal controls in terms of learning and memory.

How depressed patients think about information appears to determine many aspects of their problems in learning and remembering information. In concept learning, depressed patients develop overgeneralized hypotheses and do not "appreciate" disconfirming feedback. Their thinking appears to be global or over-generalized and therefore, their processing of new information results in the formation of poorly elaborated weak memory traces. This can also be seen in how they process emotionally salient vs. "neutral" information and other stimulus characteristics that should ordinarily lead to effective information processing.

2. Studies of dementia

Patients with progressive idiopathic dementia (most probably of the Alzheimer's type) have classically been diagnosed on the basis of the cognitive state that they present clinically. These patients demonstrate profound impairments in learning and memory as well as other kinds of cognitive operations. We have now described more fully the characteristics of the cognitive dysfunction in these patients and defined some of the mechanisms that determine their learning/memory failure. In a study of a large series of early stage Alzheimer's patients, we have demonstrated that information is lost from memory relatively rapidly, that immediate memory is relatively unimpaired, and that any type of learning/memory operation that requires the establishment of permanent trace events is dramatically disrupted. These impairments are due in large part to processing or acquisition deficits which then result in weak trace formation and therefore, failures to remember. Cognitive theorists have argued for a distinction between semantic memory (that is, the repository of information about how knowledge is organized or structured) versus episodic memory, or the memory for ongoing, recent events. Although these two kinds of memory have been viewed traditionally as being separate and distinct, we have found that there is an important linkage between the two, one that defines in large part the characteristics of the memory impairment in Alzheimer's patients. We have shown that the extent to which Alzheimer's patients have access to structures in semantic memory is the extent to which they are relatively unimpaired on many tasks of learning and memory. These results can have important implications both diagnostically, in distinguishing this group of patients from other groups, as well as for potential treatment strategies.

Cholinergic drug treatment of progressive dementia patients has proved to produce weak enhancement of some components of information processing.

It would appear that those patients who are least cognitively impaired are most likely to benefit from such treatment. In contrast, vasopressin-treated progressive dementia cases have shown temporary marked changes in learning and memory, particularly in those subjects who can access previous knowledge better following such drug treatment.

The mechanisms and determinants of the cognitive impairments in depression and dementia have allowed us to devise strategies to distinguish between these two groups clinically. Three dimensions that are particularly useful in distinguishing the cognitive impairment in depression from that of the progressive idiopathic dementia patient are: (1) that of automatic versus effortful processing, (2) the degree to which effort is extended in accomplishing tasks, and (3) the extent to which the information being processed is either random or highly structured.

3. Learning disabilities

We have examined cognitive changes in children with various forms of learning disability. Two groups of these children have been studied: in one, hyperactivity is part of the syndrome; in the second, there is no evidence of hyperactivity or generalized retardation. All of these children demonstrated dramatic impairments in learning and memory that resemble the kinds of cognitive losses seen in some groups of adults. The resemblance is closest to depressed patients, but it also mimics the kinds of cognitive changes that are produced by drugs which disrupt cholinergic and noradrenergic activity. Thus, both hyperactive and learning-disabled children demonstrate impairments in effortful processing of information, whereas automatic processing is left relatively intact. In fact, on our incidental learning paradigms, these children are indistinguishable from normal controls. Both groups of children are also impaired in those characteristics of cognition that require the imposition of organization in memory. These results are being prepared for publication, and a review article will be submitted to the Psychological Bulletin.

4. Pharmacological studies of cognition

In both patient groups and unimpaired subjects, we have found that cholinergic antagonists and agonists produce cognitive effects that are qualitatively different from those of drugs that have their major action on catecholamine activity (either as agonists or antagonists). A series of studies has been completed demonstrating the role of neuropeptides, such as synthetic vasopressin-like substances, in enhancing aspects of learning and memory. The enhancement occurs not only in cognitively impaired depressed patients but in unimpaired subjects and in patients with progressive idiopathic dementia as well. The general pattern of these findings implies that different neurotransmitter systems and/or neurochemical mediators may be involved in the regulation of various aspects of the acquisition, retention, and retrieval of information.

We have now completed a series of cholinergic trials in Alzheimer's patients and have demonstrated that: (a) cholinergic antagonists, such as scopolamine, mimic many of the characteristics of progressive idiopathic dementia; and (b) combinations of cholinergic agents produce small but reliable enhancements of some aspects of learning and memory in such patients. The amount of enhancement in learning and memory is limited by the degree of cognitive intactness of the

patients. While alcohol has been viewed traditionally as one type of pharmacological manipulation that reliably produces learning and memory impairments in man, recent work from our laboratory (in collaboration with NIAAA) has demonstrated that post-processing manipulation, including treatment with alcohol, can produce some enhancement in learning and memory. This paradoxical improvement, as well as that evident in the effect of vasopressin on reversing retrograde amnesia following administration of ECT, implies that it is important to continue to explore the biological events that succeed information processing.

B. Autonomic nervous system activity in attention and psychopathology

The central focus of this research is the role of attentional processes and autonomic nervous system (ANS) functioning in psychopathology, especially schizophrenia. Studies are directed toward several basic issues: (1) the nature of the attention and ANS dysfunction, (2) the diagnostic specificity of the dysfunction, (3) state vs. trait issues, (4) the neurobiological basis of attention and ANS functioning.

In previous research, we found that a pattern of poor attention as indexed by slow reaction time (RT), high ANS activity, slow adaptation and habituation, and a sluggish ANS response to stress were associated with a schizophrenic diagnosis and poor prognosis. In ongoing analyses of a more recent study, we have confirmed most of these findings for the schizophrenic group as a whole compared to controls. Further analyses with respect to clinical ratings and symptom patterns are underway. Preliminary analyses have shown that patients without Schneiderian first rank symptoms and who also have normal CT scans are less deviant in ANS base levels and reactivity than patients with either or both of these features. In a current study on schizophrenia, a major aim is to compare several RT methods of assessing attention deficits, some old and some new to this study, in an attempt to define more precisely the nature of attention impairments in schizophrenia. ANS activity is recorded during some of these procedures in order to investigate hypotheses about the role of ANS activity in attention impairments. These methods have been used in two studies on nonpatient males who were selected on the basis of extremely good or poor scores on either a continuous performance task (CPT) or pendulum eyetracking performance, two tests on which schizophrenics show marked impairments. Poor CPT subjects showed increasing RT impairment with increases in stimulus and response uncertainty and other evidence of disproportionate impairment in reacting to unexpected stimuli. Subjects with poor eye tracking were impaired most when there was uncertainty about the time of the onset of the critical stimulus. This suggests that eyetracking and the CPT are tapping different aspects of attention.

Diagnostic specificity of the measures is being investigated by testing patients with a major depressive illness, patients with severe obsessive-compulsive neuroses, and adults with a history of infantile autism as part of the same protocol being used with schizophrenics.

In a previous study, many of the variables which predicted clinical outcome in schizophrenics were independent of clinical state, suggesting that they may represent traits. In addition, in a previous study from this laboratory on twins, many of these variables showed a significant genetic influence. State influences are being studied in two experiments. In one, the subjects are patients diagnosed as multiple personality. Nine patients and two controls have

been tested in four to five sessions in a design which enables comparison of within personality with between personality variations for each subject. Results to date show that a majority of the subjects exhibited significant between personality variations in RT. Analyses of ANS data are in progress. In another ongoing study, women are tested in the premenstrual and follicular phases of their menstrual cycle. Some of the women are selected on the basis of regularly developing severe affective symptoms during the premenstrual period. This study may allow us to develop ANS markers for affective states. In a third study related to state influences on ANS recording during rest, a series of simple tones and a two-flash discrimination procedure are done on normal volunteers when they are either supine or standing. It is known that this postural change doubles the level of plasma norepinephrine and increases base levels of heart rate and other ANS variables. ANS activity has been found to be positively correlated with habituation rate and good two-flash discrimination. This is a pilot study to test the effect of manipulations of ANS activity, without confounded changes in attention or distraction, and if it is successful, it could have wider application in testing hypotheses about the effects of "arousal" in behavior.

Neurobiological bases of attention and ANS activity are studied by investigation of the effects of drugs with fairly specific actions and by a correlational approach. In a study published last year, we found that dextroamphetamine, in sufficiently high doses, produces ANS changes in normal men which closely resemble those found in schizophrenics. This suggests a role of the activity of either dopamine or norepinephrine in this ANS pattern. Clarification of the influences of these two catecholamines may come from comparison of the effects of pimozide, a dopamine receptor blocker, and prazosin, a norepinephrine antagonist, on ANS activity in schizophrenics. A recent study compared the effects of an MAO type A inhibitor (clorgyline) with clomipramine, which blocks serotonin reuptake, in obsessive-compulsive patients. Although only clomipramine was clinically effective, both drugs had similar effects in reducing electrodermal activity, increasing heart rate, and reducing heart rate variability. However, clomipramine had stronger effects especially on heart rate and its variability, reducing skin conductance response amplitudes and increasing the speed of habituation. The latter result is interesting because slow habituation has been implicated as an etiological factor in theories of obsessive compulsive neurosis. Since few, if any, drugs have completely specific effects, a variety of treatments will have to be investigated to specify the neurobiological bases of the ANS variables.

C. Individual differences in the study of heredity and environment in schizophrenia

The project is composed of the following studies:

1. An intensive multidisciplinary study of a family with monozygous quadruplets (daughters) concordant as to schizophrenia but discordant as to severity and outcome. The first study of this family was completed and published in book form in 1963. We have continued our contacts with this family to follow the clinical course of these women and to see how the course is related to earlier and current life experiences. A second intensive multidisciplinary study of these women was completed, so far as data-gathering was concerned, in June of 1981.

This study includes a number of the same variables that were examined in the quadruplets in the late 50's (e.g., CPT, reaction time, autonomic nervous system arousal and habituation) and adds a considerable number of new methods and techniques for assessing behavioral and biological factors. Among the new methods are the CAT scan, the PETT scan, far-field brainstem auditory evoked potentials, cerebral mapping of various EEG components and measures of many biogenic compounds from blood, urine, and CSF whose existence was unknown or for which no link to psychopathology had been established twenty years ago. The focus of this effort remains the same as it was in 1963: to help illuminate the differences in the severity of illness among these women. We have added, where possible, studies of the mother of the quadruplets, of the husband of the one who has been married, and of the two sons who have issued from that marriage. This additional information may also shed some light on possible genetic factors in schizophrenia. The data have been analyzed fully and are being prepared for publication at this time.

2. Studies of adoptees with schizophrenic parents and their biological and adoptive families

This represents a completion of work done with a cohort of adoptees and controls that were obtained in Denmark. Although a considerable portion of this work has been published, much of the psychological information was not analyzed. This is in the course of being completed at this time. In addition, a reanalysis of the original case material has been completed and submitted for publication.

3. A study of children of schizophrenic and control parents reared in town or kibbutz in Israel

This study, begun in 1965, has involved a multidisciplinary (psychological, psychiatric, neurological) examination of a cohort of 50 children with schizophrenic parents together with 50 matched controls. Half of each group were raised on a kibbutz, and half were raised with their nuclear families in cities and towns in Israel. The children have been seen twice: once in 1966-7, when they were about 10 years of age, and once in 1973, when they were about 17. Aside from several reports of the neurologic findings (suggesting more "soft" signs in the index cases) none of the work has been published. Major progress has been made this year: (1) A series of manuscripts has been prepared describing available aspects of the 1966 and 1973 studies. In addition, major portions of the data have been analyzed. These efforts will form the basis of a comprehensive report to be published in a single issue of the Schizophrenia Bulletin. (2) A third study of over 90 percent of the original Israeli cohort has now been completed, and the data are being analyzed. The results are startling, since they suggest that being raised in a kibbutz environment is more likely to lead to major psychopathology, given a schizophrenic diathesis, than is growing up in the nuclear family within a city environment.

The objectives of all of these projects are to understand how hereditary and environmental factors interact to make for schizophrenic outcomes of varying types and degrees.

D. Cognitive, perceptual, and nosological studies in depression

This project has involved a variety of studies, of which six are currently active in the laboratory. All are at the stage of completed data collection and are also at or near the completion of data analysis. Progress over the past year will be summarized below, while more detailed descriptions appear in the individual project reports.

1. Psychomotor and psychosensory symptoms in affective illness

Data have been collected on a total of 111 patients with affective illness, partial complex epilepsy, and hypertension (controls). Preliminary analyses indicate that affective and epileptic patients are indistinguishable in frequency of certain kinds of visual, auditory, visceral, and cognitive illusions and hallucination, while paroxysmal motor phenomena are present only in the epileptic group. The relationship of such symptomatology to underlying personality factors and life course of illness in the affective group is now being analyzed.

2. Cognitive processing of emotional and neutral verbal stimuli in depression

Depressed patients were found to discriminate between highly emotional and neutral stimuli in a manner similar to normal controls. However, memory performance demonstrated a different pattern of remembering of high vs. low emotional, and high vs. low concrete stimuli for the two groups. These differences have been interpreted as demonstrating relatively shallower processing of semantic properties of material by depressed subjects. A manuscript describing this work has been sumitted for publication.

3. Hypothesis testing in depression

Depressed subjects have been found to perform poorly relative to normal controls in an abstract reasoning task necessitating formulation and testing of solution hypotheses. Two types of deficits, inability to sufficiently narrow down sets of possible problem solutions and perseveration on disconfirmed hypotheses both contributed significantly to the depressive performance deficit and distinguished depressed from normal subjects. While elementary functions of memory, attention, and logic appeared to be intact, these could not be coordinated efficiently in the context of problem solving in the depressed group. A manuscript has been submitted for publication.

4. Lateralized hemispheric function in depression

Depressed female subjects have been found to show right hemisphere advantage in a verbal processing task which normally displays left hemisphere advantage. Since such a task is an obligatory left hemisphere task in normals, these results suggest shifts of hemispheric function as a concomitant of the depressed state. Further studies involving male depressed subjects and depressed-recovered patients are now being planned. A manuscript has been submitted for publication.

5. Relationship of cognitive deficits to diagnostic sub-type and neuroendocrine changes in depression

Twenty-seven depressed patients have been tested with a battery of learning and memory protocols. Degree of memory impairment was not related to presence vs. absence of DSM III melancholia, or to normal vs. abnormal TSH response to TRH challenge. However, while patients with normal cortisol suppression following dexamethasone administration displayed the usual depressive deficits, dexamethasone escapers did not. Cognitive performance was related to degree of hopelessness in the suppressor, but not the escaper, group. While these preliminary results suggest that dexamethasone response may discriminate cognitively from non-cognitively related depressions, more data are needed before any firm conclusions can be drawn. This work continues at the Psychiatric Institute of Washington, D.C. with the collaboration of Dr. Steven Targum, formerly of NIMH. These results have been presented at the 1982 meeting of the American Psychiatric Association.

6. Atypicality in primary depressive illness

The results of a pilot study investigating life course of illness and biological measures in typical vs. atypical depressed patients has been published. In collaboration with Dr. Robert Post of the Biological Psychiatry Branch, NIMH, efforts are now under way to enlarge the original sample of subjects, develop prospective methods of quantifying atypicality, and investigating atypicality in relation to long-term outcome following treatment.

ANNUAL REPORT OF THE LABORATORY OF SOCIO-ENVIRONMENTAL STUDIES
NATIONAL INSTITUTE OF MENTAL HEALTH
October 1, 1981 through September 30, 1982
Melvin L. Kohn, Ph.D., Chief

It has been a bittersweet year for the Laboratory. ADAMHA's Reduction-in-Force was seriously disruptive. As indirect results, Leonard I. Pearlin transferred his research to the University of California and Joanne Miller took a position as Associate Director for Sociology at the National Science Foundation. Pearlin's departure means the end of the Laboratory's program of research on the social-structural sources of stress. Miller's departure slows down the pace of comparative analysis of Polish and U.S. data on job conditions and psychological functioning, but, fortunately, she is able to continue as a Guest Worker on a one-day-a-week (plus evenings and weekends) basis.

This has also been an immensely productive year. Progress has been made on both the Polish and the Japanese replications of our U.S. studies of job conditions and psychological functioning. Moreover, we have this year completed the analyses and writing for a book that incorporates twenty years of the Laboratory's research on social stratification, job conditions, and psychological functioning. We have also made significant progress on the next phase of our research program, the analysis of the impact of social-structural conditions (as they operate through the educational system and the family) on the personality development of children. In particular, considerable progress was made on the analysis of educational experience and children's personality development.

SOCIAL STRATIFICATION, JOB CONDITIONS, AND PSYCHOLOGICAL FUNCTIONING

The initial objectives of the Laboratory's research on job conditions and psychological functioning were to answer two questions: What is the relationship of social stratification to psychological functioning? To what extent does this relationship result from the greater opportunity to exercise occupational self-direction enjoyed by men of higher social position? Data to answer these questions were collected in 1964, when structured interviews were conducted with a sample of 3101 men, representative of all men employed in civilian occupations throughout the United States. These interviews were conducted to Melvin Kohn and Carmi Schooler's specifications by the National Opinion Research Center (NORC) of the University of Chicago. The original analyses, published in 1969, were based on these cross-sectional data, utilizing the most advanced methods of statistical analysis then available -- exploratory factor analysis and analysis of variance. These methods do not take measurement error into account, of even greater importance, they assume unidirectional effects of social stratification on occupational conditions and of occupational conditions on psychological functioning.

NORC subsequently conducted ten-year follow-up interviews, again to Kohn and Schooler's specifications, with a randomly selected one-fourth of the men who had participated in the original survey. In the meantime, powerful new methods of statistical analysis -- confirmatory factor analysis and linear structural-equation causal analysis -- had been developed. Using the longitudinal data and the new methods of analysis, Melvin Kohn and Carrie Schoenbach have now readdressed the original questions. The new methods, as applied to longitudinal data, permit much more accurate measurement of key concepts. Moreover, they permit the analysis of reciprocal causal effects. In the new analysis, Kohn and Schoenbach assess as reciprocal the relationships between social-stratification position and occupational self-direction and between occupational self-direction and psychological functioning, testing empirically issues that previously were treated only by a priori argument.

As in the original analysis, the investigators find consistent relationships of social-stratification position with values and orientations: The higher men's social-stratification positions, the more likely they are to value self-direction, for themselves and for their children, and the more likely they are to hold self-directed orientations to self and society. The reanalysis finds that the magnitudes of these correlations are considerably larger than the earlier methods of analysis had indicated. Moreover, the pattern of correlations holds not only for values and the particular facets of orientation originally examined, but also for more fundamental dimensions of personality -- self-directedness of orientation, distress, alienation, and ideational flexibility.

The current analyses confirm, too, that the psychological impact of social-stratification position is substantially attributable to three occupational conditions that are determinative of the opportunity to exercise self-direction in work -- the substantive complexity of the work, closeness of supervision, and routinization. This conclusion is based on an empirical assessment of the reciprocal relationship between occupational position and occupational self-direction. Each affects the other strongly, with education affecting both. As a result, the correlation of occupational position with occupational self-direction is near unity. Thus, a basic tenet of the original interpretation, that there is a close relationship between social-stratification position and occupational self-direction, is confirmed. Despite this high correlation, though, it is possible (by disaggregating social-stratification position and occupational self-direction into their component concepts) to distinguish the psychological effects of social-stratification position from those of occupational self-direction. These analyses demonstrate that the psychological impact of social-stratification position (and of its components, education and occupational position) is attributable, in very substantial degree, to occupational self-direction.

The original analysis posited that the experience of occupational self-direction must actually affect values and orientation. That

proposition was both the crucial element in the entire interpretation and -- since it was based only on a priori argument -- the most questionable. Now it is possible to assess the reciprocal relationships between occupational self-direction and the several facets of values, orientation, and cognitive functioning. In every instance, occupational self-direction does have a causal impact on psychological functioning. In many instances, the relationship is reciprocal, with values, orientations, and cognitive functioning also affecting the exercise of self-direction in work. The interpretive chain is now complete: Social-stratification position affects and is affected by occupational self-direction, occupational self-direction affects and is affected by psychological functioning. Moreover, occupational self-direction, ideational flexibility, and a self-directed orientation are intertwined in a dynamic process through which the individual's place in the stratification system both affects and is affected by his personality.

In these analyses, Kohn and Schoenbach have also re-evaluated the processes by which education affects values and orientation. Contrary to the investigators' earlier expectations, ideational flexibility does not play a substantial intervening role in the process by which education affects values and orientation. Instead, ideational flexibility is more affected by, than a determinant of, self-directed values and orientations. This being the case, another hypothesis -- that ideational flexibility plays an intervening role in the process by which occupational self-direction affects values and orientations -- also falls by the wayside. The investigators conclude, instead, that the effects of both education and occupational self-direction on self-directed values and orientations are predominantly direct and, furthermore, that the effects of education and of occupational self-direction on ideational flexibility are in part indirect, through self-directed values and orientations.

A further purpose of this analysis is to address questions about social class similar to those asked about social stratification. By social stratification, the investigators mean the hierarchical ordering of society; by social classes, they mean groups defined in terms of their relationship to ownership and control of the means of production. Since social class represents a theoretically powerful alternative conceptualization of the socio-economic organization of industrial society, they ask the same questions about social class that they ask about social stratification: What is the relationship of social class to values, orientations, and cognitive functioning? To what extent does this relationship result from the greater opportunity to exercise occupational self-direction enjoyed by those who are more advantageously situated in the class structure?

The analysis shows that social class does have an appreciable psychological impact, similar to that of social stratification. Moreover, occupational self-direction plays a crucial role in explaining the psychological impact of social class, just as it does in explaining the psychological impact of social stratification. The psychological effects

of social class examined in the analyses prove to be mainly a function of the varying degrees of occupational self-direction enjoyed by men variously situated in the class system.

HOUSEWORK AND PSYCHOLOGICAL FUNCTIONING

On the hypothesis that the psychological impact of the conditions of work encountered in household work would be similar to those of job conditions encountered in paid employment outside the home, Kohn and Schooler included in the 1974 follow-up survey a set of questions about the nature of the actual work performed by the respondents in taking care of their households. Insofar as possible, these questions exactly parallel those asked about work performed in paid employment. The household-work questions were asked not only of the men in the follow-up study, but also of their wives, who were independently interviewed.

With this information, Carmi Schooler, Melvin Kohn, Joanne Miller, and Karen Miller have in past years developed measurement models of the basic conditions of work encountered in housework and have done multiple-regression analyses of the relationships between housework and psychological functioning. These analyses provide evidence that women's psychological functioning is related to the housework they do. Ideational flexibility is positively related to doing substantively complex housework and negatively related to doing housework that is heavy, dirty, repetitive, or in which the woman believes she is likely to be held responsible for things outside her control. Self-directedness of orientation is positively related to doing substantively complex housework and negatively related to doing dirty housework. Distress is related to doing housework under felt pressure of time or under circumstances where a woman believes she may be held responsible for things outside her control. All these findings are consonant with the possibility that women's housework affects their psychological functioning.

Although it is plausible that the multiple-regression findings result in substantial part from housework actually affecting psychological functioning, the direction of causal effects is far from certain. It could be argued that the relationship between housework and psychological functioning results mainly from women's personalities shaping the way they do their housework. To appraise causal direction, it is necessary to use linear structural-equation modelling, to estimate possible reciprocal effects. After considerable difficulty in developing statistically sensible models, the investigators finally succeeded in testing appropriate models. These analyses support the belief that the relationships found between household work and psychological functioning result largely from housework affecting psychological functioning. In particular, doing substantively complex housework results in increased ideational flexibility and a more self-directed orientation, while doing heavy housework results in a diminution of both.

Corroborating earlier multiple-regression analyses for men, linear structural-equation analyses of men's housework and psychological functioning indicate that it is not the substantive complexity but the heaviness of men's housework that is of central importance for their psychological functioning. The psychological effects of heavy housework are more similar to those of substantive complexity in paid employment than to those of heavy work in paid employment. Why doing heavy housework should have such psychological effects is, however, unclear. One possibility is that physical exertion in an off-the-job context facilitates both intellectuality and a sense of efficacy, while sedentariness is detrimental to both.

Equally puzzling is why the substantive complexity of men's housework ceases to be significantly related to their psychological functioning when other housework conditions are statistically controlled. The most likely explanation of this finding lies in the differential meaning of housework for men and for women. For many women, household work has much the same demand characteristics as does the work required in paid employment, thus, housework is a structural imperative whose psychological effects are similar to those of the structural imperatives of paid employment. For most men, in contrast, housework exerts no such imperative and thus does not have psychological effects similar to those of paid employment. If this interpretation is correct, the absence of significant relationships between the substantive complexity of men's housework and their psychological functioning, even though unanticipated, can be seen as consistent with the general interpretation. It may only be when it is imperative that work demands be met that the conditions of work have their usual psychological effects.

In a more extended analysis of the nature of housework, Schooler, Karen Miller, and Joanne Miller have also carried out descriptive analyses of the work performed for the household and for pay, comparing husbands to wives and employed wives to full-time homemakers. In addition, husbands' and wives' perceptions of their household responsibilities were examined. These descriptive analyses show that the working conditions of housework do not differ greatly from the working conditions of the average paid job. As expected, however, there are marked sex differences in spheres of responsibility for, and performance of, particular household tasks. Men's housework tends to be limited to household repairs, heavy work, and balancing the checkbook, while women are believed to be responsible for, and actually do, a wider range of household tasks.

EDUCATIONAL EXPERIENCE AND CHILDREN'S PSYCHOLOGICAL DEVELOPMENT

The purpose of Karen Miller, Melvin Kohn, and Carmi Schooler's analysis is to examine the processes by which students' educational experiences, particularly the degree of self-direction they are able to exercise in their educational endeavors, affect their psychological functioning. Data for this analysis were collected in the 1974 follow-up survey, when one pre-selected child of each father in the sample was interviewed. The interview schedule for these "children" -- aged 13 to 25

-- contains an intensive battery of questions about the current educational experiences of all those respondents still in school. These questions are designed to parallel those previously found to be powerful for analyzing occupational experience, focusing on such dimensions of the educational experience as its substantive complexity, how closely it is supervised, time-pressure, and the like. The intent is to see whether the concepts and methods developed for the study of occupational conditions can be applied as well, and with similar results, to the study of educational conditions.

Part of this year's work was devoted to refining the measurement model of "educational self-direction," a preliminary version of which was developed in 1980-81. "Educational self-direction" is directly analogous to occupational self-direction; it is defined to mean the use of initiative, thought, and independent judgment in schoolwork. In the refined model, the substantive complexity of schoolwork and the absence of close supervision by teachers are the major indicators of educational self-direction.

Another part of this year's work was the development of a measurement model of intellectual flexibility for students, a model exactly comparable to that earlier developed for adult men and women. The model is not intended to reflect innate intellectual ability or scholastic achievement, it does reflect students' actual intellectual functioning in a situation (i.e., the interview) outside the school context. Intellectual flexibility is conceived as incorporating two factors -- perceptual and ideational flexibility -- with the latter being the major focus of the research.

The main thrust of the analysis thus far has been to develop and test a causal model of the reciprocal effects of educational self-direction and the ideational component of intellectual flexibility. The investigators encountered considerable difficulty in attempting such an analysis without longitudinal data. One tremendous advantage for this particular analysis, however, is the availability of information about the students' parents' psychological functioning. This means that, in examining the relationship between educational self-direction and any facet of psychological functioning, it is possible to statistically control both parents' levels of functioning. Thus far, one provisional model has been developed -- a model of the reciprocal effects of educational self-direction and ideational flexibility, taking into account parental ideational flexibility, age, sex, and school grade of the child, the extent to which the child's school courses are compulsory or elective, and pertinent social characteristics of the child and the family.

Preliminary results suggest that educational self-direction, in particular the substantive complexity of schoolwork, has a decided causal impact on students' ideational flexibility. It thus appears that, even in competition with the powerful genetic and environmental effect of parents' intellectual functioning and the powerful developmental effect of age, substantively complex schoolwork increases a student's ideational flexibility. During the coming year, the investigators will refine this

model and further test this provisional conclusion. In addition, they will develop measurement models of other aspects of psychological functioning, such as self-directedness of orientation, distress, and values, testing their reciprocal relationships with educational self-direction.

THE POLISH REPLICATION

The main purpose of this inquiry has been to see whether the interrelationship of social stratification, job conditions and psychological functioning found in analyses of the U.S. data is similar in a socialist society. Three principal co-investigators, Kazimierz Slomczynski, Krystyna Janicka, and Jadwiga Koralewicz-Zebik, carried out in 1978 in Poland a precise replication of the survey originally conducted by Kohn and Schooler in 1964 in the United States. After the data had been collected, coded, and edited in Poland, Slomczynski brought them to NIH, where he, Joanne Miller, and Melvin Kohn have been analyzing them. Previous Annual Reports reviewed their development of methods designed to assure cross-national comparability of indices and their analysis of two of the central questions of the Polish replication: Do people's positions in the system of social stratification bear the same relationships to their values and orientations in socialist Poland as in the capitalist U.S.? If so, do these relationships result from the greater opportunities for occupational self-direction enjoyed by men of higher social-stratification position? As discussed in last year's Annual Report, the answers to both questions are positive with respect to values and social orientations. But higher social stratification position does not make for greater self-confidence in Poland (as it does in the United States), at least in part because occupational self-direction does not affect self-confidence in Poland.

In further research this year, Joanne Miller, Slomczynski, and Kohn have been investigating how job conditions and education relate to effective intellectual functioning for men in early, middle, and late stages of their careers. There are several reasons to expect variability in these relationships. Although previous research demonstrates that both education and conditions of work that require initiative and independent judgment promote intellectual flexibility, intellectual functioning may be less responsive to social conditions in men's later years. It is also possible that successive birth cohorts may respond differently to educational and occupational conditions, because of changes in the nature of work and of educational experience. These alternative possibilities cannot be resolved with either the American or Polish data alone. But, by making cross-national comparisons, some assessment of the regularity of these relationships can be made.

The analysis is divided into two major phases: assessing the comparability of measured concepts across the life-course as well as cross-nationally, and testing causal models. The first phase of the analysis has just been completed.

Intellectual flexibility is measured by five indicators reflecting intellectual performance in the interview situation: two tests of problem-solving ability, the Embedded Figures Test requiring that one differentiate figure from ground, the tendency to systematically agree with agree-disagree questions, and the interviewer's rating of the respondent's intelligence. The investigators developed measurement models to test whether these indicators can be used to measure intellectual flexibility in both countries and at all stages of the life-course. Their analyses demonstrate substantial comparability regardless of nationality or age.

However, two small differences are found. The tendency to agree with agree-disagree questions is a stronger indicator of (lack of) ideational flexibility in the United States than in Poland. This finding is consistent with the knowledge that in Poland the interview situation is culturally defined as a formal interaction, Polish respondents may try to avoid disagreement in such a context. Thus, in addition to reflecting intellectual functioning, this indicator also appears to reflect a particular cultural orientation towards the interview situation -- making "agree set" a weaker indicator of intellectual flexibility in Poland. Second, Polish interviewers appear to evaluate the overall intelligence of respondents on somewhat different criteria depending on the age of the respondent. For older men, the interviewer's assessment of intelligence seems to be strongly influenced by the respondents' performance on the cognitive tests. For younger men, factors other than demonstrated performance influence the interviewer's rating. It is not possible to determine if these exogenous criteria are legitimate indicators of intelligence or whether they reflect irrelevant characteristics such as physical attributes. In any case, no such pattern is found in the American data. In all, though, while some differences in measurement exist, they are of minimal importance. The correlations among scores based on several alternative models of ideational flexibility are high and the alternative models all fit the data reasonably well.

Having established confidence in the measures indexing intellectual flexibility, the investigators next assessed the average competencies of workers at different stages of the life course. Three age groups were examined: men 30 years of age or younger, men 31 to 45 years of age, and men aged 45 or older. Both the American and Polish studies find that, on average, older men are less intellectually flexible than are younger men. Further analyses will explore whether this results from the greater education of younger workers, from differences in the job conditions encountered by younger and older workers, from other related social processes, or from aging itself.

In the second phase of this analysis, Miller, Slomczynski, and Kohn will investigate how job conditions and education affect the intellectual flexibility of workers at different stages of career. The analysis will focus on three job conditions reflecting occupational self-direction: substantive complexity, closeness of supervision, and routinization. As with the index of intellectual flexibility, the investigators have

developed age-specific measurement models of these concepts in each country. Here, too, while there is some variation in the structure of the models across age groups and between countries, the correlations among differing models are all high and they all fit the data reasonably well. Both in Poland and in the United States, young workers are found to be less self-directed in their jobs than are middle-aged and older workers. However, the advantage of age subsides after the middle years.

THE JAPANESE REPLICATION

Another major replication of the U.S. research on job conditions and psychological functioning has been conducted in Japan by Atsushi Naoi and Ken'ichi Tominaga of the Department of Sociology of Tokyo University. Data collection took place during the summer and fall of 1979. At that time, a probability sample of more than 800 employed men was interviewed, using a questionnaire containing all the questions necessary for indexing job conditions and those aspects of psychological functioning measured in the original study. Data-analysis began in October, 1980, when Naoi came to the Laboratory as a Visiting Scientist to work colloboratively with Carmi Schooler. Initially, confirmatory factor analysis was used to develop measurement models for occupational self-direction, intellectual flexibility, parental values, and several facets of self-conception and social orientation. These measurement models proved to be generally similar to those that had previously been developed for American men.

This year saw the completion of one of the major goals of the study -- the analysis of the causal relationship between occupational self-direction and psychological functioning. The central result is the generalization to Japan of the American findings. Occupational self-direction is found to have significantly positive effects on ideational flexibility, personally responsible standards of morality, and trust, and to have significantly negative effects on authoritarian conservatism, idea conformity, fatalism, and self-deprecation. In Japan, as in the United States, occupational self-direction leads to ideational flexibility and a self-directed orientation to self and society. As in the U.S., ideational flexibility and a self-directed orientation also reciprocally affect occupational self-direction.

The analyses also show a more extensive relationship in Japan than in the U.S. between position in the organizational structure and psychological functioning. The causal models strongly suggest that in Japan ownership, hierarchical level, and bureaucratization positively affect self-esteem. All three aspects of organizational position lead also to greater authoritarian conservatism. The comparatively greater authoritarian conservatism of Japanese in advantaged organizational positions may be both a means of asserting their authority and a reflection of the attitudes of others. Comparison of the Japanese and American measurement models indicates that authoritarian conservatism in Japan is marked by a higher degree of obeisance and respect for those in authority than is authoritarian conservatism in the United States. In Japan, hierarchical

level also results in more idea-conformity and in standards of morality that emphasize pragmatic obedience rather than acceptance of personal responsibility for one's actions. This tendency of high position to lead to a conformist orientation indicates that "favored" occupational conditions do not universally lead to a self-directed orientation.

During the year, preliminary analyses of the relationships of social stratification to self-conception and social orientation in Japan were also carried out. These analyses confirm that, with one exception, the relationships of social stratification with social orientations and self-conceptions are similar in sign to those found in cross-sectional analyses of data from the United States, albeit perhaps smaller in magnitude. The exception is that, in Japan, there is little if any relationship between social-stratification position and anxiety, it may even be that men of higher social-stratification position are more anxious than are men of lower position.

Analyses were also begun on the role of occupational self-direction in explaining the relationship of social stratification to psychological functioning in Japan. Because the analyses are still in process, the conclusions remain provisional. At this stage, occupational self-direction appears to explain, in substantial part, the relationships of social stratification to ideational flexibility, to social orientations, and to those aspects of self-conception that are related to social stratification.

Taken together, these analyses substantially reinforce the impression that, although meaningful cross-national differences exist, the Kohn-Schooler interpretation of the interrelationships of social stratification, job conditions and psychological functioning is generalizable to Japan. Although the Japanese analyses have not been completed, there is now evidence that in Japan and the U.S. psychological constructs have essentially the same measurement characteristics and are (with the exception of anxiety) similarly related to social stratification. In both countries, the magnitudes of these relationships are substantially reduced when occupational self-directon is statistically controlled. In each country, occupational self-direction can be shown to affect those aspects of psychological functioning that are related to social stratification. Thus in Japan, as in the United States, the individual's position in the social-stratification system affects his psychological functioning in large part because it embodies systematically different conditions of work that profoundly affect his personality.

STATISTICAL METHODS

The key analytical instrument of research in the Laboratory is a computer program written and maintained by Ronald Schoenberg called MILS (Multiple Indicator Linear Structural Analysis). Schoenberg has significantly expanded the capabilities of this instrument during the year. He developed a new technique for the analysis of latent product variables (i.e., latent variables that are products of other latent variables), which

allows for the estimation of parameters of quadratic equations in which the variables contain measurement error. The estimation of the parameters of models with product latent variables had previously been impossible. Schoenberg incorporated this technique into the MILS computer program. Two Generalized Least Squares (GLS) methods for the solution of linear structural equations have also been added to MILS, in addition to the Maximum Likelihood (ML) method previously available. GLS requires fewer statistical assumptions and therefore allows the estimation of models in which the available data fail to conform to the multi-normality asssumption required for the ML method. Other investigators have become interested in MILS and the improvements added to it. About twenty universities have requested and have received this program, and are using it in their research.

During the year, Schoenberg began the extension of latent variable techniques to the analysis of observed polytomous variables (i.e., variables that are measured in a usually finite number of discrete categories). Methods were developed to analyze Binomially-distributed and Poisson-distributed data. Observed variables of these types present major difficulties when the usual methods of statistical analysis, which assume that data are normally distributed, are applied. There have been recent advances in computer technology and statistics, however, that provide the basis for the solution of these problems. These new procedures have been applied to problems under investigation in this and other Laboratories and they show an improvement over traditionally used techniques.

In addition to helping the laboratory keep up to date on research methods -- through personal consultation as well as by means of a formal weekly class on statistical methods -- Schoenberg has assisted investigators in other Laboratories and in other parts of the Government in updating and improving their research methods. He has assisted in the development of methods for analyses of the acute effects of alcohol on intellectual functioning in a study supported by NIAAA and NIMH, of the effects of parents' behavior on infants' behavior for an investigator in NICHD, of a measurement model of commuters' attitudes for an investigator in DOT, of a model of alcohol use, aging, and mental ability for an investigator in the National Institute of Aging, and of a model of health care for an investigator in the Health Care Financing Administration.

Proposed course of further research

As is evident above, the analysis of the Polish and Japanese replications is incomplete, with much more to be done. The analysis of the relationship between education and psychological functioning, too, is far from complete. In addition to completing these analyses, the investigators intend to embark on an analysis of the processes by which parents' values and practices affect the values and personality development of their children. There are data in both the U.S. and Polish studies with which to carry out such an analysis. A provisional model of these processes has been developed and preliminary analyses have been undertaken.

Annual Report for 1982

Laboratory of Brain Evolution and Behavior

National Institute of Mental Health

Paul D. MacLean, M.D., Chief

Our field Laboratory is engaged in two quite different lines of research. One group of workers is involved in neurobehavioral investigations, while the other, comprising the Unit for Research on Behavioral Systems (URBS), is primarily concerned with ecological factors that affect the behavior of large populations of animals. Both groups have been singularly fortunate in receiving long-term support for research that looks at the whole organism, as well as its component parts.

The neurobehavioral section of the Laboratory moved to the Poolesville installation in 1971, to have greater latitude for conducting research on the two older evolutionary formations of the forebrain. In its progressive evolution, the primate brain has expanded to a great size while retaining structural and chemical features that reflect an ancestral relationship to reptiles, early mammals, and late mammals. For nearly a hundred years it has been the clinical view that the striatal complex (representing the oldest evolutionary formation of the forebrain) is primarily involved in motor functions. This view has prevailed in spite of the recognition that destruction of large parts of the complex may result in no motor deficits. In past neuropsychological research it has been traditional to test animals in situations in which they manipulate inanimate objects. At the Poolesville facility, it is the purpose to learn whether or not experiments on animals living under seminatural conditions and interacting with other animals might reveal cerebral functions that would otherwise not be apparent. In regard to the striatal complex, this approach has been rewarding because it has demonstrated in animals as diverse as reptiles and primates that a large subdivision of the complex is essential for integrating displays used in animal communication. Concerning the anatomy and chemistry of the pallidum, it is of special interest that an improved histochemical stain and quantitative measurements have shown that the iron content of this structure is markedly higher in female rats than in males. Moreover, the research has demonstrated lawful, marked fluctuations in the iron content during the estrous cycle and pregnancy. In this year's work it was found that the injection of an iron chelator into the third ventricle interrupts the estrous cycle, possibly through an effect on the iron in the tanycytes in the region of the median eminence. Such findings are to be considered in the light of the statistic that iron deficiency is the most prevalent nutritional disorder of the human population.

The foregoing behavioral approach has also been productive in investigating the functions of the limbic system, which represents an inheritance from

early mammals. In the evolution from reptiles to mammals three distinctive innovations were the development of (1) nursing in conjunction with maternal care, (2) audiovocal communication for maintaining maternal-offspring contact, and (3) play behavior. In addition to confirming findings that the limbic cingulate cortex plays an essential role in maternal care, our work in an earlier project demonstrated that this cortex is also involved in the expression of play behavior. Based on preliminary findings in a current project it now appears that the cingulate cortex is also involved in the production of the isolation call (separation call) of mammals. Since the isolation call served originally to maintain maternal-offspring contact, it probably ranks as the most primitive and basic mammalian vocalization. If the present experiments on squirrel monkeys are confirmed in these and other species, it will indicate that three forms of behavior that characterize the evolutionary dividing line between reptiles and mammals have depended in part on the differentiation and expansion of cortex forming the newest part of the limbic system.

Earlier work in our own Laboratory had demonstrated that rodents entirely devoid of the neocortex from birth were remarkably capable of performing various forms of rodent-typical behavior. Although providing a quasinatural habitat, our own experiments did not permit observations on the coping ability of animals living in complex environments containing several breeding pairs of animals. The workers in URBS have attempted to conduct such experiments, using room size habitats with many nest boxes and operant devices for dispensing water and food. There is a computerized system for recording the location and activity of all animals. In an attempt to achieve uniformity in the amount of brain loss, the experimental subjects were offspring of dams treated on the 15th day of pregnancy with 20 mg/kg of methylazoxymethanol acetate (MAM). Through methylation this alkylating agent affects DNA and causes necrosis of dividing cells. Such treatment not only severely affects the development of the neocortex, but also the limbic cortex. In addition to the neocortex, the limbic cingulate cortex, in particular, is greatly reduced in area and distorted in appearance. This condition would help to explain the deficits in maternal behavior of the treated group. Compared with the control group, the experimental animals were only 10% as effective in rearing pups from the initial litters. Other differences were increased aggression and less neophobia in the experimental groups. Although the present investigation did not make it possible to answer the specific question regarding the neocortex, it illustrates the usefulness of such kinds of experiments for obtaining information relevant to phylogenetic and ontogenetic questions, as well as the general problem of mental retardation.

This year saw the completion in URBS of two long-term experiments on populations of mice and rats that were begun eight years ago. The work is relevant to the world-wide problem of overpopulation and has the two-fold purpose of identifying factors that either contribute to or alleviate the ill effects of crowding. The outcome of the experiments in regard to four main questions will be briefly summarized as follows:

1. In an earlier mouse study, it had been found that an increase in population to eight times optimum led to a cessation of mating and breeding, followed by extinction of the population. In the studies recently completed,

it was the purpose to learn whether allowing a two-fold increase in time for the population to double would help to counteract the ill effects of over-crowding. This modification appeared to be beneficial with respect to mating and bearing young. After the population reached eight times optimum, however, there were no surviving young because of interference with maternal care.

2. Earlier studies had indicated that bilaterally symmetrical habitats might be preferable to those of radial design for alleviating the ill effects of crowding. This prediction proved to be wrong. Regardless of design, the more dominant animals lived on one side of the habitat and the less dominant on the other. The situation was reminiscent of the times when the poor and the well-to-do "lived on opposite sides of the tracks." The important factor in habitat design, it turns out, is to provide plenty of routes for escape from interactions with other individuals.

3. In earlier experiments there were indications that mixing animals of different genetic strains contributed to the deleterious effects of crowding. The results of using interbred strains in the mouse and rat studies just completed indicate that there is less strife under these conditions.

4. Finally, a main purpose in the last rat study was to learn whether or not the opportunity for cooperative behavior would make it possible for animals to live more amicably under conditions of crowding. The answer to this question was in the affirmative. In the bilaterally symmetrical habitats designed for them, both the control and cooperative animals developed two opposed groups, one on each side of the habitat. Unlike the animals that learned to cooperate, the controls persisted in maintaining a dividing "Berlin wall" by plugging the central portal that connected the two sides.

The obvious parallels between what occurs in human affairs and in the experiments described here attest to the usefulness of population studies on animals for clues to factors contributing to, or alleviating, the deleterious effects of crowding.

Annual Report of the Laboratory of Cerebral Metabolism
National Institute of Mental Health

Louis Sokoloff, M.D., Chief

October 1, 1981 through September 30, 1982

The Laboratory of Cerebral Metabolism still consists of two Sections with independent research programs in the area of biochemistry of the nervous system. Although their programs are quite distinct in their orientation, the two Sections share equipment, facilities, and in some cases methodology, particularly with respect to analytical biochemical procedures. The Laboratory suffers severely from inadequate space, particularly the absence of a laboratory/conference facility and a room to house some of the computer facilities. Space restrictions result in allocations of facilities usually on the basis of expediency rather than rational organization of resources. The Laboratory, nevertheless, maintains an active research program which has achieved and retains a position of worldwide respect.

Section on Developmental Neurochemistry
Louis Sokoloff, M.D., Chief

This Section continues its research along three lines: 1) the refinement and extension of the deoxyglucose method for the measurement of local cerebral glucose utilization and its application to various physiological, pharmacological, and pathological conditions; 2) intermediary energy metabolism of the central nervous system; and 3) the development and applications of a method to measure local rates of protein synthesis in the central nervous system.

The deoxyglucose method was originally developed for use with ^{14}C, and the resolution of the method with this isotope is approximately 50 μm. The resolution is limited by the autoradiographic procedure, but the principles of the method are applicable to finer resolution, even microscopic. Because of the weaker energy of the β-radiation from ^{3}H and the availability now of ^{3}H-sensitive film, it is possible to achieve finer resolution with [^{3}H]deoxyglucose. Dr. Francesco Orzi has been adapting the method for use with [^{3}H]deoxyglucose. He has calibrated a set of [^{3}H]methylmethacrylate standards to quantify the autoradiography and is in the process of completing a series on normal control animals studied with [^{3}H]deoxyglucose for comparisons with the normal values previously determined with [^{14}C]deoxyglucose. The resolution is clearly improved and may now be down to 10 μm. In fact, in some experiments, single cells have been seen in the autoradiographs, as, for example, lower motor neurons in the ventral horn of the cat cervical cord.

Experience with the deoxyglucose method has led to the recognition that it breaks down under conditions of severe hypoglycemia or hyperglycemia, far beyond the normal range of blood glucose concentration. Because a number of physiological, pharmacological, and pathological conditions are characterized by marked changes in the blood glucose level, it became important to analyze and adjust the method for this phenomenon. The problem has been localized to changes in the "lumped constant" and rate constants, components of the operational equation of the method. The "lumped constant" has been redetermined over the entire range of arterial plasma glucose concentrations from 40-600 mg% in studies carried out over the past two years, first by Sumio Suda and Franz Schuier, and in the past year by Francesco Orzi and Diana Dow-Edwards. Drs. Orzi and Dow-Edwards are now completing

measurements of the rate constants, and are studying the effects of plasma glucose concentration on local cerebral glucose utilization.

Bruce McFarlin and Charles Goochee have been working on the three-dimensional reconstruction of the brain's local glucose utilization by computerized image-processing. This would allow examination of all the structures of the brain from any plane of section of the brain from a single experiment in which the brain was sectioned either coronally, horizontally, or parasagittally. This project is temporarily suspended because of Mr. McFarlin's recent departure, but it will be reactivated when a replacement programmer is recruited.

Previous studies with the deoxyglucose method on the effects of dopamine agonists demonstrated metabolic activation in all components of the dopaminergic extrapyramidal motor system. Surprisingly, no effects were seen in the mesolimbic dopaminergic system. These studies were carried out acutely with single doses of d-amphetamine or apomorphine. Drs. Orzi and Dow-Edwards have examined the effects of chronic d-amphetamine administration by osmotic pumps. One week of administration results in increases of glucose utilization in the nucleus accumbens, in contrast to the lack of effect of a single dose. These results are consistent with the hypothesized basis of amphetamine psychosis, which is considered by some to be a model of schizophrenia.

The applications of the deoxyglucose method have been extended into the field of neuroendocrinology. Dr. Dow-Edwards has studied the effects of cretinism on local cerebral glucose utilization. In rats radiothyroidectomized at birth but allowed to reach adult age, there are marked and widespread decreases in glucose utilization throughout the brain, but the cerebral cortex, particularly the primary sensory cortical regions, e.g., visual, auditory, and somatosensory, are by far the most affected. The results are in keeping with neuroanatomical studies which indicate that the neuropil of the cerebral cortex is underdeveloped in cretinism. Drs. Linda Porrino and Hiroki Namba are carrying out studies designed to relate local changes in cerebral glucose utilization to sexual behavior in the female rats. Local cerebral glucose utilization varies with the stages of the estrous cycle. Physiological doses of estradiol administered by chronic implants stimulate glucose utilization in a number of hypothalamic nuclei. Progesterone reverses this effect of estradiol while having relatively little effect of its own. The increases in glucose utilization in the hypothalamic nuclei caused by estradiol coincide with an increase in sexually receptive behavior. Drs. Massako Kadekaro and Paul Gross, in collaboration with Dr. Juan Saavedra, are studying local cerebral glucose utilization in a genetic strain of rats with a defect in vasopressin synthesis (Brattleboro DI rat). They have found that despite the deficiency in vasopressin synthesis, the hypothalamic-hypophysial tract, especially the posterior pituitary, is metabolically activated. Replacement treatment with vasopressin, which reverses the symptoms of diabetes insipidus exhibited by these animals, does not reverse the metabolic activation. The mechanisms underlying the metabolic activation are under study.

Dr. Charles Kennedy and Dr. Masanori Ito, in collaboration with many investigators of the Laboratory of Neuropsychology and the Sleep Laboratory under Dr. Christopher Gillin, have completed their studies of local cerebral glucose utilization in slow-wave (non-REM) sleep in monkeys. Their results indicate a diffuse uniform reduction in glucose utilization throughout the central nervous system. No structures, not even those proposed as hypnogenic centers which when activated

supposedly depress other regions of the brain and produce the state of sleep, showed anything other than decreased metabolism. These results support, therefore, a chemical basis of slow-wave sleep acting throughout the nervous system rather than the hypnogenic center theories of sleep.

Dr. Carolyn B. Smith, with the assistance of Drs. Malin Ingvar and Philippe Maeder, is continuing her studies on the influence of aging in the central nervous system of rats. In previous work she found age-dependent selective reductions in glucose utilization in specific regions of the brain. Particularly affected were the components of the primary visual and auditory components, similar to effects seen following blockade of the primary sense organs. These results suggested that some of the central nervous changes in aging might be secondary to sensory deprivation or deafferentation. Also affected by aging were components of the dopaminergic nigrostriatal pathway. These are structures in which glucose utilization can be activated by dopamine-agonists, such as d-amphetamine and apomorphine. In studies still in progress Dr. Smith has found decreased responsivity of these dopaminergic structures to apomorphine with age. These results suggest a loss of functional dopamine receptors in the nigrostriatal system with aging and may offer a basis for the development of senile parkinsonism.

The major research effort of the Section is directed at the development of a method for the measurement of local rates of protein synthesis in the nervous system. This technique is designed to be an autoradiographic procedure and utilizes some of the principles developed for the deoxyglucose method, but it is far more complex because of the special biochemical properties of the protein-synthesizing system. The development of the method and some of its applications are under the direction of Dr. C.B. Smith. The problems are the possibility of intracellular compartmentation of the amino acids used for protein synthesis and the recycling of amino acid derived from protein breakdown, and she has designed progressively more complete but complex kinetic models to take these phenomena into account. She has also derived the appropriate operational equation to go with each model. The completion of the development of the method awaits only the clarification of which of these models is most appropriate. During the past year, an experimental procedure has been designed that will provide this clarification. The procedure consists of a programmed infusion of [^{3}H]amino acid to provide a constant arterial concentration for a variety of intervals, a different animal for each interval. At the end of the interval the animal is killed, the brain removed, and the tRNA-amino acids extracted from the brain tissue. The tRNA-amino acid must be purified by a variety of steps and then deacylated, and the free [^{3}H]amino acids are then dansylated with [^{14}C]dansylchloride. The specific doubly labeled dansylated amino acid is then separated by high pressure liquid chromatography, and the $^{3}H/^{14}C$ ratio measured in a liquid scintillation counter. The amino acid in the plasma is similarly treated to determine the $^{3}H/^{14}C$ ratio. This procedure will provide the rate constants for the equilibration of the precursor pool amino acid with that of the plasma and from the equilibrium $^{3}H/^{14}C$ values of the precursor amino acid compared to that of the plasma amino acid will also provide the evidence for the presence or absence and the degree of recycling of amino acid from protein breakdown. This procedure consists of a number of complex chemical procedures, and Dr. Smith has during the past year systematically worked out all the details of each step. Several experiments have already been carried out, but the brains derived from these experiments have not yet been fully analyzed.

This final series of experiments on the modelling is required only to achieve absolute quantification of the rates of protein synthesis. Even without them, the first model designed by Dr. Smith can provide a reasonable estimate of the rates of protein-synthesis which are adequate to examine some biological processes. For example, Dr. Smith has used the method to study regeneration in the hypoglossal neural pathway. Section of the hypoglossal nerve in the rat is followed within 4 days by increased protein synthesis in the hypoglossal nucleus which persists until the nerve has regenerated fully and has become functional. The increased protein synthesis is preceded by an increased rate of glucose utilization in the nucleus. The mechanism of the increased energy metabolism and its relationship to the increased protein synthesis and process of regeneration is under study. Last year the method was used to study plasticity in the visual system of the newborn monkey. Acute patching of one eye had no effects on protein synthesis anywhere in the brain, but a chronic patch of approximately 3 weeks resulted in a decreased rate of protein synthesis in the lateral geniculate laminae normally served by the deprived eye. Inasmuch as the protein synthesis required for axonal sprouting and growth of the geniculostriate pathway resides in the cells of the lateral geniculate, this result provides evidence that the loss of ocular dominance columns in the visual cortex following unilateral eye patching is the result of reduced protein synthesis and axonal growth in the pathway for the deprived eye. Dr. Smith is continuing studies of this phenomenon in monkeys to determine the age range of plasticity, the effects of reverse patching, etc. The method is also being used in studies of slow wave sleep in the adult monkey to determine whether there are changes in protein synthesis in any specific structures. The results thus far indicate decreased protein synthesis throughout the brain, except for 3 regions with increased protein synthesis in sleep -- i.e., locus coeruleus, area postrema, and substantia innominata.

Drs. Elaine Kaufman and Thomas Nelson are continuing their biochemical research on the origins, disposition, and functional significance of γ-hydroxybutyrate in brain. This compound is normally present in brain, but when administered in pharmacological doses, it produces a trance-like state and a marked generalized depression of cerebral energy metabolism. Reports in the literature suggested that the behavioral effects of the compound are blocked or diminished by naloxone; Drs. Kaufman and Nelson in collaboration with Dr. Gregory Crosby, a previous Guest Worker in the Laboratory, have found that naloxone also antagonized its metabolic effects. These results suggest that opiate receptors may be in some way involved in the mechanism of its behavioral and metabolic effects. Drs. Kaufman and Nelson have also made significant progress in elucidating the biochemical origin of the compound. γ-Hydroxybutyrate had been believed to be derived from the metabolism of γ-aminobutyric acid, but Nelson and Kaufman have recently found that γ-hydroxybutyrate is present in even larger amounts in extraneural tissues, like kidney or liver, which lack glutamic decarboxylase, the enzyme responsible for the formation of GABA. In recent studies with labeled substrates they have obtained evidence that γ-hydroxybutyrate is derived from GABA in kidney also, but the GABA is generated from polyamines, such as putrescine, and/or arginine. A series of enzyme reactions involving pyridine nucleotides are responsible for this alternative pathway for GABA formation. Studies are continuing on the properties of the enzymes involved in γ-hydroxybutyrate synthesis and oxidation.

The deoxyglucose method depends on the trapping of the label in deoxyglucose in deoxyglucose-6-phosphate or any other compound derived from it. There have been recent reports in the literature that deoxyglucose is also incorporated into

glycogen. Dr. Thomas Nelson has systematically examined this reaction and has found that deoxyglucose is indeed incorporated into glycogen but relatively slightly. In the course of a typical deoxyglucose experiment less than 1-2% of the label is in glycogen; the remainder is in deoxyglucose-6-phosphate. With the usual quantitative autoradiographic procedure employed, this incorporation into glycogen is of no significance to the results. There have, however, been attempts to increase the resolution of the method by fixation of the tissue. Fixation results in major loss of the water-soluble deoxyglucose-6-phosphate while the labeled glycogen is retained. Under these circumstances the deoxyglucose method does not measure glucose utilization, but is only a stain for the presence of glycogen.

Section on Myelin Chemistry
Marian W. Kies, Ph.D., Chief

Research of the Section on Myelin Chemistry has two major themes: (1) basic proteins of central and peripheral myelin; and (2) mechanisms of pathogenesis in an experimental autoimmune disease of the central nervous system. These diverse areas of research require professional and technical personnel trained in neurochemistry and/or immunology. Over the years, our efforts to recruit and train such a staff have resulted in a steady growth in our research capabilities and in our contributions to research on myelin and demyelinating disease. The summary will be presented under these two themes:

Basic Proteins of Central and Peripheral Myelin.

The major antigens involved in autoimmune pathology of the nervous system are myelin basic protein (BP) and P-2. BP, which was first isolated from central nervous tissue by Dr. Kies and co-workers, was later found in peripheral myelin as well. P-2, which is usually localized in peripheral myelin, is also found in low concentrations in central nervous tissue (spinal cord) myelin. The two proteins have been the subject of many research investigations, primarily because of their ability to induce experimental allergic encephalomyelitis (EAE) and experimental allergic neuritis (EAN).

In last year's report, Dr. Martenson described the structure of P-2 as a highly ordered tertiary structure consisting of two domains each made up of five β-strands organized into two anti-parallel sheets; according to his prediction, the two domains would be joined by the formation of a parallel β-sheet. He has now completed his predictions on the structure of P-2. At the biennial meeting of the International Society for Neurochemistry (Sept., 1981), he was gratified to learn that the "active" site of P-2 (the peptide sequence responsible for EAN induction) was in a region of the molecule which he had predicted would be exposed and not buried in the compact core of the protein. This location of the active site would ensure its availability for interaction with sensitized cells.

Peptic fragments prepared from rabbit myelin basic protein and characterized by Dr. Martenson have been used in a collaborative study with Dr. Walter Moore of the University of Sydney. NMR spectra of these peptides were analyzed and the different threonine (Thr) γ-CH_3 resonances assigned to specific Thr residues in the protein. The data indicate that the various Thr residues in BP are physicochemically non-equivalent. Thus, when BP undergoes conformational changes as, for example, when it goes from the aqueous phase into lipid complex formation, it

should be possible to use the Thr residues as "reporter" groups to determine which parts of the molecule undergo conformational transitions.

A third project pursued by Dr. Martenson resulted in identification of three new in vivo phosphorylation sites: Ser-7, Ser-56, and Ser-113 as well as confirmation of two sites (Thr-96 and Ser-163) reported earlier. This work is particularly significant in that it adds a new dimension to studies from other laboratories showing rapid in vivo incorporation of P-32 into myelin basic protein. Additional studies on phosphorylation of BP in vivo have been carried out by Dr. Martenson in collaboration with Dr. Harish Agrawal at Washington University in St. Louis.

Mrs. Deibler has developed an HPLC technique for purifying highly basic fragments of BP for use in Dr. Driscoll's studies on mechanisms of enhanced transfer of EAE. The purification of peptide fragments of BP has enabled us to establish beyond any doubt that the active region in guinea pig (GP) BP responsible for disease induction is the same part of the molecule which induces a proliferative response in BP-sensitized cells in vitro, and further, that this site is also required for enhanced transfer. Linkage of these three activities (in guinea pigs) to the same peptide sequence -- i.e., the nine amino acids located in the peptide chain at positions 113 to 122 will help us eventually to identify the T-cell subsets involved in the disease reaction.

Mrs. Deibler has just finished a study on the sequence of guinea pig BP. Although this protein was the first BP to be isolated and characterized, it has never been completely sequenced, whereas BP from several other species (bovine, human, rat, rabbit) has been sequenced. Information on the sequence of GP BP accumulated gradually as a consequence of our preparation and characterization of specific peptic and V-8 protease fragments of BP. We identified the location of specific residue additions and deletions in the peptide chain by comparing the fragments of GPBP with the corresponding peptides of bovine BP. Just recently, in collaboration with Dr. Krutsch of the NIAID, Mrs. Deibler has completed the entire sequence of GPBP.

In addition to facilitating the interpretation of our own biological data on the basic proteins of myelin, these studies have also been useful to others in their research on EAE and EAN.

Mechanisms of Pathogenesis in E perimental Autoimmune Disease of the CNS.

The technique of enhanced transfer has been used to study mechanisms of pathogenesis in EAE. Enhanced transfer refers to the induction of EAE in naive animals by transfer of BP-sensitized donor cells after the cells have been incubated in vitro with antigen (BP). In vitro exposure to antigen enhances the efficiency of the cells at least 100-fold. The technique (first described by Dr. Driscoll in 1979) is now widely used in EAE as well as in other autoimmune diseases, such as thyroiditis.

Of particular importance to EAE is the knowledge that successful transfer of delayed type hypersensitivity (DTH) requires that the cell donor and recipient must share at least some genes in the immune region (I region) of the major histocompatibility complex (MHC). The I region of the MHC and the Ia (I region associated) cell surface (membrane) antigens which are encoded there play a central role in control of DTH responses, such as EAE. By using antisera to Ia antigens

to destroy specific cell populations, Dr. Driscoll has been able to gain a better understanding of the roles played by these various cell populations in EAE. It is clear that BP-sensitized cells found in different compartments of the animals are expressing different amounts of Ia antigen on their surface -- Strain 13 lymph node cells clearly have little Ia on their membrane whereas peritoneal exudate cells have significant amounts of Ia antigen on their membrane.

In addition to further studies on the role of Ia antigen in EAE, Dr. Driscoll has been investigating the development of chronic EAE in adult Strain 13 guinea pigs after they receive a suboptimal number of BP-sensitized cells and are subsequently sensitized with whole guinea pig cord. This variant of EAE is considered to be a better model for multiple sclerosis than acute EAE induced by a single inoculation of BP.

In studies on acute EAE, he discovered that a suboptimal transfer of cells ($\sim$ 20% of the usual dose) protected the recipients against subsequent induction of EAE by an injection of BP in CFA. Dr. Driscoll's careful analysis of this disease suppression convinced him that suppressor cells, as such, were not responsible for "interference" with sensitization. Because we have always been interested in the possible existence of a second encephalitogen in whole tissue he tested the ability of suboptimal cell transfer to suppress whole tissue sensitization. As we expected, the disease induced by whole tissue was more difficult to overcome than that induced with BP. What we did not expect was that the protected survivors would develop chronic EAE, similar in every respect to the chronic EAE induced in juvenile Strain 13 guinea pigs by other investigators. Dr. Alvord's histologic examination of coded specimens clearly links the development of large purely demyelinating lesions to this induction technique (i.e., suboptimal transfer followed by whole tissue sensitization). The major importance of this observation is that it establishes the fact that BP-sensitized cells play a key role in chronic EAE just as they do in acute EAE, and, further, provides a tool for analyzing the mechanism of demyelinating plaque formation in experimental animals which has previously not been available. Understanding the mechanism of plaque formation could ultimately provide a logical approach to the prevention of plaque formation in multiple sclerosis.

EAE in Lewis rats differs in many respects from EAE in guinea pigs. One major difference is that enhanced transfer of EAE can be achieved with BP-sensitized rat spleen cells which have been cultured with concanavalin A (Con A). Con A in culture has no such effect on guinea pig lymphoid cells. Dr. Namikawa has found that the culture supernatant derived from Con A treated rat spleen cells is capable of enhancing transfer of EAE with BP-sensitized LNC. (The spleen cells need not be sensitized with BP). LNC cultured with Con A do not produce an active supernatant unless they have previously been cultured in medium containing fetal calf serum. The reactions which are involved in the production of active factor(s?) as well as in the cellular differentiation which is required for response to the factor(s?) are obviously complex. A positive result (i.e., successful transfer) tells us only that some limiting step in the process has been overcome. Nevertheless, the observation that enhancement can be mediated by a soluble factor is a very important contribution to our eventual understanding of the mechanism of enhanced transfer.

Annual Report of the
Laboratory of General and Comparative Biochemistry
National Institute of Mental Health
October 1, 1981 to September 30, 1982
Giulio L. Cantoni, M.D., Chief

Fiscal year 1982 has seen continued progress in the research programs of the Laboratory of General and Comparative Biochemistry in spite of the limitation on our creativity imposed by the long hiring freeze, RIF and other administrative restrictions.

The Laboratory research efforts are organized around the four senior investigators: S. Harvey Mudd, Werner A. Klee, Carl R. Merril and Giulio L. Cantoni. Although the four groups are independent, there is considerable interaction and scientific interchange within the Laboratory, since the four groups share the same or similar technologies and scientific approach. The research programs of Drs. Mudd and Cantoni, in particular, are interrelated and mutually complementary.

In the last few years, Dr. Cantoni's group has directed its attention to the physiological correlates and significance of biochemical methylation reactions and their controls. It has become increasingly clear since its discovery thirty years ago that S-adenosyl-L-methionine (AdoMet) is a compound of unique biochemical versatility and extraordinary metabolic significance. The participation of AdoMet as substrate: a) in a great many and varied reactions involving synthesis of compounds of metabolic and physiological importance, or b) for the post-transcriptional modifications of nucleic acids and proteins, and as an allosteric effector in a number of complex and very interesting biochemical systems must require a control mechanism for the cellular regulation of its utilization.

As the result of studies by Cantoni and his collaborators as well as those of other investigators, it is now thought that the intracellular ratio of adenosylmethionine/adenosylhomocysteine might play a key role in the regulation of biological methylations. In addition to its role as an inhibitor as well as a product of biological methylations in vertebrates, S-adenosyl-L-homocysteine (AdoHcy) is the only source of homocysteine (Hcy) and the principal source of adenosine (Ado). A supply of Hcy is an absolute requirement for the biosynthesis of cystathionine and cysteine, via the transsulfuration pathway, and for the regeneration of tetrahydrofolic acid from methyltetrahydrofolic acid via the B_{12}-dependent synthesis of methionine catalyzed by methyltetrahydrofolic acid-homocysteine methyltransferase.

In eukaryotes, AdoHcy is metabolized through a single pathway by the enzyme S-adenosyl-L-homocysteine hydrolase (AdoHcyase), first discovered in this Laboratory by de la Haba and Cantoni. More recently, Cantoni and collaborators have returned to a study of AdoHcyase and examined a relatively large number of adenosine analogs for their ability to interact with AdoHcyase as inhibitors, alternate substrates or both. Among these, 3-deazaadenosine (DZA) and 3-deazaaristeromycin (DZA-Ari) are particularly interesting because while they exhibit some common features, they differ significantly both from a biochemical and a biological standpoint.

DZA and DZA-Ari appear to interact with a single enzymatic target: AdoHcyase. Neither is attacked by adenosine deaminase or phosphorylated by adenosine kinase. DZA-Ari is a potent reversible inhibitor of the enzyme and in vivo its administration results in large accumulation of AdoHcy that reflects the rate of biological utilization of AdoMet for transmethylation reactions. By contrast, DZA can function both as a strong inhibitor and a good substrate for the enzyme. Thus, in vivo its administration results both in the accumulation of AdoHcy and in the intracellular formation of an AdoHcy congener, deazaadenosylhomocysteine (DZA-Hcy). An interesting consequence can be predicted from our knowledge about the mode of action of DZA and DZA-Ari. While the accumulation of DZA-Hcy upon administration of DZA indicates that the inhibition of AdoHcyase has not been complete and that some homocysteine has been generated by cleavage of AdoHcy, it might be expected that formation of Hcy might be completely inhibited by DZA-Ari. This would secondarily result in inhibition of cystathionine synthesis and in accumulation of methyltetrahydrofolic acid.

Kim, Aksamit and Cantoni have found that administration of DZA-Ari results in a very profound, possibly complete, inhibition of AdoHcyase and inhibits the growth of mouse macrophage. The cytostatic effects of DZA-Ari are a consequence of this inhibition since when deprived of an adequate supply of homocysteine the cells are unable to recycle methyltetrahydrofolic acid and regenerate tetrahydrofolic acid for use in de novo synthesis of purines and pyrimidines needed for nucleic acid biosynthesis. In support of this interpretation, it was found that micromolar quantities of homocysteine, folic acid and pyrimidines will "rescue" DZA-Ari inhibited cells and allow resumption of growth.

Aside from the intrinsic biochemical interest of these observations, they could have important clinical significance. It has been shown that in a variety of clinical conditions, AdoHcyase activity becomes severely reduced. Thus, in patients suffering from adenosine deaminase deficiency disease the AdoHcyase of erythrocytes is completely, and irreversibly inhibited by the accumulation of 2'-deoxyadenosine, a suicidal inhibitor of AdoHcyase. 9-β-D-Arabinofuranosyladenine (Ara-A) supplemented with an inhibitor of adenosine deaminase such as erythro-9-(2-hydroxy-3-nonyl)-adenine (EHNA) or deoxycoformycin, is currently being tested in a series of clinical trials in cancer chemotherapy. It has been suggested that inhibition of AdoHcyase that is seen as a side effect of Ara-A + EHNA administration might contribute both to the toxic and the therapeutic effects of this compound by virtue of the fact that accumulation of AdoHcy would inhibit a variety of biologically important transmethylation reactions. While this possibility undoubted must be considered, we would suggest that a more important consequence of the inhibition of AdoHcyase in vivo might be its effects on the sulfur conservation pathway. It would, therefore, be of great interest to determine if the blood level of methionine is abnormally low in patients suffering with untreated Ado deficiency or in patients undergoing chemotherapy with Ara-A plus an Ado deaminase inhibitor. Dietary supplementation might be indicated in these clinical situations where the patients might be close to, or actually in, a negative nitrogen balance. On the other hand the trapping of tetrahydrofolic acid as methyltetrahydrofolic acid might contribute to the oncostatic activity of Ara-A by limiting the amount of tetrahydrofolic acid available for purine and pyrimidine biosynthesis. It should also be pointed out that the combination of Ara-A, EHNA and methotrexate should be avoided since the methyltetrahydrofolic acid rescue therapy would fail due to insufficient homocysteine.

Homocysteine lack would also limit the synthesis of cystathionine. This amino acid has been found to be present in the brain and especially in the pineal gland in high level. While no specific functions have been assigned to brain cystathionine, the possibility remains that it might have a hitherto unrecognized role as a neuro-transmitter, or that its absence might cause neurological deficits with a long time delay.

Accumulation of AdoHcy, whether brought about by DZA or DZA-Ari results in vivo in the inhibition of a number of transmethylation reactions such as phospholipid methylation. Biologically, while DZA and DZA-Ari have some similar biological properties, such as potent antiviral action, they differ in one very interesting aspect.

Only DZA can bring about inhibition of mouse macrophage chemotaxis, a finding that has been ascribed by Aksamit and Cantoni to a specific inhibition by DZA-Hcy of one or more reactions that are essential for the chemotactic response. Phospholipid methylation, that had earlier been thought by others to be necessary for the translation of the chemotactic stimulus into cellular movement is equally sensitive to inhibition by DZA and DZA-Ari. It was concluded by Aksamit, Backlund and Cantoni, therefore, that the incorporation of the methyl group of methionine into phosphatidylcholine, is not directly related to macrophage chemotaxis, since this reaction is not inhibited specifically by DZA. In another biological system it was found that DZA administration inhibits the synthesis of a few proteins required for viral oncogenic transformation. It was of interest to determine if the inhibition of chemotaxis by DZA could be ascribed to the specific inhibition of one or more proteins important for the chemotactic response. It was found that indeed DZA but not DZA-Ari will cause the inhibition of the synthesis, at least one, possibly a few proteins identified by two-dimensional gel electrophoresis. Neither DZA nor DZA-Ari have a major effect on protein synthesis. However, with a number of other compounds, a striking correlation was found between the ability to inhibit the synthesis of a few proteins and inhibition of chemotaxis.

The conclusion that emerges from these results is that macrophage chemotaxis requires the continued synthesis of a small group of proteins. In order to explain by a single mechanism how the DZA and other compounds cause inhibition of chemotaxis, we propose that DZA-Hcy inhibits the synthesis a "functional" mRNA coding for one or more "chemotactic proteins." This inhibition could be due to a variety of mechanisms, one of which could be inhibition of the methylation reaction(s) required for the formation of the 5'-cap structure of mRNA. Further work will be necessary to establish the validity of the working hypothesis outlined above that assigns to DZA-Hcy an interesting and specific role as an inhibitor of nucleic acid methylation.

Klee and his colleagues have continued their studies aimed at clarifying the structure and function of opiate and related receptors. Purification of receptor components, a major goal of the group, has received an important boost from the synthesis by Rice, Jacobson and their colleagues (LC, NIAMDD) of a number of irreversible opiate ligands which form covalent bonds with recognition units of the receptors. Several of these are highly selective towards different receptor subtypes and studies with radiolabelled, irreversible, opiates suggest that specifically labelled proteins form and can be isolated and characterized.

As a result of the work on function of receptors, it has become clear that opiate receptor action involves as a minimum a receptor, or recognition protein, and an effector protein which is the GTPase turn-off protein of the adenylate cyclase complex. The recognition protein and GTPase are separated in detergent (CHAPS) extracts of membranes even though receptors in such extracts are still sensitive to GTP modulation. Thus, a third component of the system appears to be a GTP binding protein which may couple opiate binding to GTPase stimulation. Klee and Streaty have recently succeeded in developing an assay system suitable for study of the function of the several components of the opiate receptor-adenylate cyclase complex. They have shown that opiate mediated inhibition of adenylate cyclase can be induced in membranes of cells lacking opiate receptors upon fusion of such membranes with others containing opiate receptors but lacking adenylate cyclase. Thus, opiate receptors can diffuse freely within membranes and interact with adenylate cyclase from any of several sources.

Sweat and Klee have developed procedures for the preparation of cell surface membranes from neuronally derived cells in a highly enriched state. Electrophoretic analysis of such membranes shows a greatly simplified protein pattern and procedures are being developed for identifying specific receptor, ion channel and second messenger producing protein components of these membrane preparations.

Merril and collaborators have continued their studies of individuals with genetic diseases affecting the central nervous system. High resolution two-dimensional electrophoresis was demonstrated to be useful as a diagnostic aid and to identify pathophysiological alterations associated with inborn errors of metabolism. In the Gilles de la Tourette syndrome, it was used as an aid in demonstrating that the primary mutational event does not affect the hypoxanthine phosphoribosyl transferase enzyme. In the Lesch-Nyhan syndrome, it was used to identify eleven lymphocyte proteins which are altered quantitatively. It has also been used to discover polymorphic proteins in studies of families with Alzheimer's disease and Huntington's disease.

The ability to observe multiple polymorphisms in a subset of lymphocyte proteins with two-dimensional electrophoresis raises the possibility that this technique may provide a powerful tool for human genetic linkage studies. Genetic modelling studies predict that 200 polymorphisms would provide a human genetic linkage map which would cover almost 70% of the genome. Such a map will prove useful in any disease study in which a genetic component may be present. By surveying 400 proteins from skin fibroblast cells from individuals with one, two, and three copies of Chromosome 21. It was possible to find two proteins that demonstrate gene dosage affects. One of these proteins was shown to be superoxide dismutase.

The development of techniques to detect chromosomally-integrated viral genomes, viral mRNA and virally altered metabolism in host cells has encouraged us to apply these technologies to investigate human diseases. The possibility of an association between a viral genome (integrated in the chromosome) and the occurrence of diseases inherited in a dominant manner, such as, familial Alzheimer's disease is currently being examined.

Integration of viral related nucleotide sequences in the genomic DNA of man will be studied using fibroblast cell lines established from individuals belonging to a family with histologically-confirmed Alzheimer's disease.

During the past year, work in the Section on Alkaloid Biosynthesis has con-

tinued to focus chiefly upon the aspartate biosynthetic pathway in higher plants. This pathway is the chief means of production in nature of four amino acids essential for man and other non-ruminant animals: methionine, lysine, threonine and isoleucine. The first three of these amino acids may limit the nutritive value of diets deficient in animal proteins, such as are eaten by millions, perhaps billions, of individuals in the world today. Essential amino acid deprivation during infancy has been implicated as a major cause of irreversible mental retardation. It is hoped that in-so-far as our work provides insight into how these amino acids are made and how their production is regulated and interrelated to other physiological events, rational optimization of such production may become possible.

Within this general framework, recent work by Dr. Giovanelli has now established for the first time in plants (other than the possibly atypical ripening fruits) the existence of a metabolic pathway for conversion of methylthioadenosine to methionine. The pathway has been unequivocally demonstrated both in intact Lemna and in Lemna extracts. The whole plant work has permitted preliminary assessment of the rates at which this pathway functions under a variety of physiological conditions, and investigation of the consequences of inhibition of the pathway. The in vitro work promises to be helpful in defining one or more intermediates in the overall transformation. While the implications of the existence of such a novel pathway will require much more work to be fully understood, its demonstration allows us to interpret unexpected results of isotopic labeling patterns obtained over the past years, and to conclude with assurance that methionine regulates its own biosynthesis, and that the locus of the regulation is at the step which commits both cysteine and O-phosphohomoserine to methionine synthesis, namely cystathionine γ-synthase.

The availability of Dr. Veluthambi, a Visiting Scientist whose salary is kindly being covered by another Institute, has provided an opportunity to initiate investigation of threonine synthase, the enzyme which catalyzes the conversion of O-phosphohomoserine to threonine. Partitioning of O-phosphohomoserine between threonine sysnthesis and cystathionine synthesis determines the relative rates at which threonine and methionine are made. Important results include demonstration by S-adenosylmethionine that threonine synthase can be stimulated allosterically 20-fold under suitable conditions. Further investigations of this enzyme after partial purification should greatly further our understanding of a reaction central to the aspartate pathway.

Drs. Thompson's and Datko's studies of the adaptation of Lemna to sub-lethal methionine deprivation have revealed means to dissociate the effects of methionine upon protein synthesis and upon morphogenetic events. Thereby an opportunity is created to come to understand more fully some of the chemical events controlling morphogenetic pheonomena.

Dr. Datko's studies of sulfate uptake by Lemna have revealed unexpected complexity to this area also. Two uptake systems have been demonstrated, differing in K_m, V_{max}, pH effects on activity, and, most importantly, in regulation by cystine and sulfate. It has been shown convincingly that the physiologically important system has high affinity for sulfate, is not competed for by phosphate or nitrate, and is highly susceptible to regulation so as to keep constant the amount of sulfur admitted to the plant. Analyses of the concentrations of a range of sulfur-containing metabolites in Lemna growing under steady-state conditions in the presence of a variety of sulfur sources are expected to be very

helpful in clarifying the mechanisms of several of the regulatory phenomena now under study in this laboratory.

The international questionnaire survey of patients with homocystinuria due to cystathionine β-synthase deficiency being conducted by Dr. Mudd and collaborators in a number of universities and medical centers has been satisfactorily initiated. Response to date from physicians world-wide has been gratifying. Data collection is still going on, and analyses have not yet commenced. Nevertheless, it now appears that standardized data will be obtained on as many as 350 patients, about an order of magnitude more than have been reported in any single study heretofore. These data should be powerful enough to provide statistically significant answers to many questions concerning genetically determined homocystinuria.

Dr. Cantoni has recently initiated a collaboration with Prof. R. Strom and Dr. S. Scarpa, of the University of Rome, designed to investigate the role of DNA methylation in gene expression and cellular differentiation. The collaboration will be financed predominantly by the Italian National Research Council.

Our senior scientists continue to participate actively, often at no cost to the Government, in presenting lectures at various universities, and also in national and international meetings, including the Transmethylation Conference, Seminar on Polyamines and Differentiation Processes, Two-Dimensional Electrophoresis Meeting, Pediatric Metabolic Meeting, etc.

carried out by Hill, Howarth, Keynes and others. (Note that the time-resolution of heat measurements with a thermopile is of the order of 100 msec.) We are planning to develop new methods of detecting water uptake by the nerve fiber.

B. Biochemical Studies of the Mechanism of Nerve Excitation. (J. Baumgold, P. Gallant, and I. Zimmerman)

During the course of cellular differentiation, inexcitable precursors of muscle and nerve cells--- called myoblasts and neuroblasts--- gradually acquire electrical excitability. By following the course of development of excitability, the appearance of the modification of integral membrane-proteins which subserve excitability were examined.

Myoblasts from chick skeletal muscle grown in tissue culture were used. It was found that, in the normal culture medium, electrical excitability of neuroblasts gradually develops and reaches the maximal level by about 8 days in culture. Simultaneously, the ability of the cell surface to bind saxitoxin and to respond to batrachotoxin with an enhancement of Na-ion uptake was found to develop along a similar time-course. When scorpion toxin is present in the medium, however, the time required for the full development of excitability was only 4 days in culture. Based on these and other findings, a hypothesis was proposed concerning the process of development of excitable sites in the nerve membrane. We propose that excitable-site proteins are incorporated into the membrane initially in an inactive form and later undergo a post-translational modification which convert them into an active form. Currently, we are using a monoclonal antibody directed against the excitable-site protein in order to purify it in an attempt at substantiating our hypothesis.

In a separate series of experiments, the nature of the biochemical processes underlying suppression of excitability by a rise in the intracellular Ca-ion concentration was studied. It was shown, using squid giant axons, that activation of an endogenous protease by Ca-ion is one of the major factors which leads to suppression of excitability. This protease is considered to digest and destroy membrane proteins involved in the maintenance of excitability. The present finding throws new light on the mechanism of Wallerian degeneration of nerve fibers.

C. Regulation of protein and enzyme function by modulator sites on complex carbohydrates. (A. L. Stone)

Dr. Stone was transferred from the Laboratory of Neurochemistry to this Laboratory quite recently. Her research is concerned with conformational aspects of modulation of protein function and enzyme systems by heparin-like complex carbohydrates. Heparin-like proteoglycans are present in small amount in cell membranes of most tissues, including the nervous system, and may be involved in cell migration, cell-cell recognition, and in fixing certain enzymes to cell membranes. By virtue of their strongly polyanionic nature, they are also implicated in the binding and exchange of simple and complex cations in interstitial tissues.

Recent results demonstrated for the first time that the multiple effects of heparin on antithrombin, a protein inhibitor of the clotting esterases, are

ANNUAL REPORT OF THE LABORATORY OF NEUROBIOLOGY
National Institute of Mental Health
October 1, 1981 to September 30, 1982
Ichiji Tasaki, M.D., Ph.D., Chief

The goal of the research activities in the Laboratory of Neurobiology is to elucidate the nature of the excitation processes in nerve fibers and cells on a macromolecular basis. Both physicochemical and biochemical approaches are being employed to achieve this goal. Since these excitation processes form the basis for the physiological function of the nervous system in normal as well as abnormal conditions, studies of these processes are relevant to the program of the Institute.--- "A physician without physiological knowledge is like a watchmaker who is supposed to correct the abnormal action of a clockwork without knowing the normal operating condition of the machine" (E. F. W. Pflueger, 1868)--- At present, the following three research projects are being carried out.

A. Analysis of the macromolecular structure of nerve membrane during excitation. (I. Tasaki, K. Iwasa, and P. Byrne)

In 1980, a new phenomenon was discovered in this Laboratory. We found that the process of excitation in nerve fibers is accompanied by swelling of the superficial layer of the nerve fiber. This discovery was preceded by the development of extremely sensitive devices for detecting small mechanical changes in the nerve fiber. During the past years, we have greatly improved both the sensitivity and the time-resolution of our devices for detecting minute displacements taking place at the surface of the fiber. It is now possible to record the time-course of a single transient displacement of the order of 10^{-6} cm with a time-resolution of about 10^{-3} sec. The piezoelectric device we have developed for detecting pressure changes in the nerve fiber is capable of registering changes of the order of a few dyn/cm^2 with a resoltuion of 10^{-4} sec. By using a signal averager in conjunction with our mechanical devices, an almost 100-fold increase in the sensitivity could be achieved.

We have established, by using these mechanical devices, that the outward displacement of the squid axon membrane closely follows the rising phase of the action potential and reaches its maximal value at the peak of the action potential. The peak amplitude of the displacement was fould to be 1 - 3 times 10^{-7} cm in the squid axon and approximately 10-times as large in a bundle of crab nerve fibers. Both the amplitude and the time-course of the observed outward displacement indicate that the rapid swelling of the nerve fiber during excitation is not caused by the Na-K ion-exchange associated with production of an action potential. We have accumulated experimental evidence indicating that the invasion of water molecules into the superficial layer---called "axolemma-ectoplasm complex"--- of the nerve fiber is at the base of the phenomenon of swelling. (Note that, in artificial membranes, the intramembrane water-content is a major factor which determines the membrane conductance.) The phenomenon of swelling is very sensitive to the Ca-ion concentration in the external medium.

We believe that studies of the phenomenon of swelling throw more light on the mechanism of excitation than the analyses of heat production in the nerve

mediated by way of increasing perturbation of the conformation of the protein. Thus, activation of antithrombin against an early enzyme of the clotting cascade results in the binding of the major, oligosaccharide modulator site (a hexamer that contains a unique saccharide sequence) with a concomitant, striking effect on the conformation of antithrombin. A second conformational perturbation is required to activate antithrombin against thrombin, as well as factor X_a. This change can be mediated only by the binding of octadecasaccharide or higher molecular weight fractions and appears to involve a disulfide bridge. Structure-function relations of these heparin-derived modulators from the octa- to the octadecassacharide were elucidated using newly developed conditions for far ultraviolet circular dichroism spectroscopy and optical models derived from model compounds. For the first time a disaccharide sequence has been proposed up to 6500 daltons, which includes the second binding region.

The molecular basis for the in vitro regulation of striatal tyrosine hydroxylase by heparin has also been elucidated for the partially purified enzyme. Furthermore, oligosaccharides from patients with Sanfilippo mucopolysaccharidoses have been isolated. These genetic disorders involve the accumulation of heparan sulfates resulting in atrophy of central nervous system tissue and concomitant mental retardation. We plan to test these heparan sulfate fragments as putative modulators in a developmental biological system.

ANNUAL REPORT OF THE LABORATORY OF NEUROCHEMISTRY
NATIONAL INSTITUTE OF MENTAL HEALTH
OCTOBER 1, 1981 THROUGH SEPTEMBER 30, 1982

Seymour Kaufman, Chief

During the last year, one of the major efforts of the Laboratory of Neurochemistry has been a continuation of our analysis of the regulation of the essential components of the aromatic amino acid hydroxylating systems, with emphasis on the hepatic phenylalanine hydroxylase system.

One of the reasons why the regulation of this last system is of special interest to neurochemists is that it exemplifies the dynamic metabolic interaction between peripheral organs, such as the liver, and the central nervous system. When this interaction goes awry, as it does in classical phenylketonuria (PKU), the consequences to the brain can be devastating.

We have gained important new insights into the regulation of hepatic phenylalanine hydroxylase. First, following up our previous finding that this enzyme can be activated by phosphorylation, a reaction catalyzed by cyclic-AMP dependent protein kinase, and that this process is accelerated in vivo by the administration to rats of glucagon, we have now shown that this enhanced activity of phenylalanine hydroxylase is actually expressed in the whole organism. This is the first demonstration that phenylalanine hydroxylase activity is under acute hormonal control.

We have also extended our knowledge about how the activity of this enzyme can be regulated by its substrate. In this regard, we have shown that not only is phenylalanine able to activate the enyzme, but that other large neutral amino acids, such as L-methionine, can also activate it and that these other amino acids can act synergistically with phenylalanine. These results have provided strong support for the idea that the hydroxylase has a regulatory site that is distinct from its catalytic site and that occupancy of the former site by one of a group of amino acids activates the enzyme.

We can now construct a fairly detailed picture of the acute regulation of phenylalanine hydroxylase activity and propose reasonable explanations for why this kind of regulation may be important.

It seems likely that one of the advantages of maintaining fine control over phenylalanine hydroxylase activity would be the protection that this would afford the developing brain. Even transient elevations of blood phenylalanine levels, including those that would result from the ingestion of protein, could be damaging to the neonatal brain. It is probably important, therefore, to keep these periods of postprandial hyperphenylalaninemia as brief as possible. Activation of phenylalanine hydroxylase would accomplish this.

Our work has shown that a dual mechanism probably exists by which hepatic phenylalanine hydroxylase "senses" the load of phenylalanine that it must dispose of. First, there is the direct activating effect of phenylalanine. This amino acid, working synergistically with others like methionine and tryptophan, would ensure that high phenylalanine hydroxylase activity is available when it is needed.

Secondly, there is the indirect activation of phenylalanine hydroxylase that would be mediated by glucagon. Since amino acids including phenylalanine can elicit the release of

glucagon, this mechanism, too, would provide a link between elevated blood and tissue levels of phenylalanine and enhanced activity of hepatic phenylalanine hydroxylase. The sequence of steps involved in this homeostatic mechanism would be the following: ingestion of protein; digestion of the protein resulting in increased blood levels of amino acids including phenylalanine; amino acid-induced release of glucagon from the pancreas; activation of hepatic glucagon-coupled adenyl cyclase; increased concentration of c-AMP; increased activity of c-AMP-dependent protein kinase; activation of phenylalanine hydroxylase; accelerated rate of disposal of phenylalanine; more rapid return of blood and tissue phenylalanine concentrations to the basal range.

Recently, another type of homeostatic mechanism for the control of phenylalanine hydroxylase that may also be mediated by glucagon was found. In this type of control, the steady state is disturbed not by a bolus of phenylalanine derived from the diet but by removal of about 2/3 of a rat's liver, and of course, 2/3 of the liver's phenylalanine hydroxylase molecules. We found that within 24 hours of partial hepatectomy, the remaining hydroxylase molecules had been activated. Preliminary results indicate that this activation involves phosphorylation of the hydroxylase.

We have continued our studies on pterin replacement as a therapy for variant forms of PKU that are caused by defects in BH_4 regeneration or metabolism. Last year we had shown that, contrary to the accepted dogma, pterins like BH_4 and the synthetic analogue with very high cofactor activity, 6-methyltetrahydropterin ($6MPH_4$), when given to rats at a proper dose, can enter the brain. Although $6MPH_4$ was found to enter the brain 10 times better than did the same dose of BH_4, even the latter compound crossed the blood brain barrier well enough so that it was possible to double normal BH_4 concentration in the brain.

We have now extended these findings to patients with PKU due to defective synthesis of BH_4. Two children with this inborn error of metabolism were treated with an adequate dose of either BH_4 or $6MPH_4$. With both patients, CSF levels of BH_4 or $6MPH_4$, respectively, were very significantly elevated. Administration of either of these pterins to one child corrected some of the defects in brain biogenic amine metabolism with concomitant dramatic improvement in neurological signs, whereas in the second child, the improvement in both the biochemical and neurological deficits was marginal. Significantly, in the child which showed the beneficial effects, there was evidence that the response was superior to that observed with the therapy used previously, i.e., administration of DOPA and 5-hydroxytryptophan with a peripheral decarboxylase inhibitor. It seems likely that pterin administration at the proper dose initiated as early as possible will prove to be an adequate therapy for these variant forms of PKU. In addition, our findings that these pterins can cross the blood brain barrier in humans opens up the possibility of studying the effect of pterin administration in other disorders that are believed to be caused by defects in catecholamine and serotonin metabolism, such as certain forms of depression.

We have found several different mechanisms by which the de novo synthesis of BH_4 from guanosine triphosphate (GTP) can be regulated. In brain and in neuroblastoma, there is evidence that the biosynthetic pathway is under feedback control, with exogenous BH_4 inhibiting the first enzyme in the pathway, GTP cyclohydrolase. This inhibition would explain why the urinary level of neopterin, a pterin derived from a normal intermediate in the biosynthetic pathway, is elevated in patients with PKU due to a lack of dihydropteridine reductase. These patients are unable to reduce BH_4 at normal rates so that their tissue BH_4 levels are exceedingly low. In the absence of the feedback inhibition that would normally be exerted by BH_4, neopterin levels are high. These observations, therefore, provide a reasonable mechanism for what previously appeared to be a puzzling pattern of pterin excretion in this disease.

In the pineal gland, we have found that BH_4 synthesis is down-regulated by an adrenergic, c-AMP-dependent mechanism. We are currently exploring the possibility that this regulatory mechanism also operates in the brain.

Dr. Neville and his colleagues have continued their studies of the use of receptor-mediated transport to develop highly specific drugs. During the last year they have prepared conjugates of ricin and monoclonal antibodies against T cells. In the presence of lactose, this conjugate reduces the T cell population of murine donor bone marrow by greater than 95% without affecting the viability of the marrow stem cell population. By this treatment, the immunological memory of the donor marrow is obliterated. This treated marrow, therefore, can be infused into a irradiated recipient without causing graft-versus-host disease. As a result, a new marrow can be reconstructed. A similar anti-T cell conjugate directed against human T cells has been constructed and has been shown to have similar properties.

The availability of these conjugates should facilitate the use of bone marrow transplantation across major histocompatibility barriers in humans. If this can be accomplished, treatments for a variety of fatal autoimmune diseases will be available by providing a new marrow for an irradiated recipient.

Dr. Nash and his colleagues continue to study the genetic recombination responsible for the integration of a bacterial virus genome into its host chromosome. Previous studies indicated that a viral protein is responsible both for breakage of DNA and its subsequent rejoining to form recombinants. This hypothesis is now supported by the demonstration of specific cleavage of DNA by purified protein at the site of recombination. Study of the topological change in circular DNA that results from recombination has shown that this process is highly ordered. More specifically, no random unwinding of DNA occurs in the time between the breakage of DNA and its final resealing.

Annual Report of the Laboratory of Neurophysiology
National Institute of Mental Health
Edward V. Evarts, M.D., Chief
October 1, 1981 -- September 30, 1982

The goal of the Laboratory of Neurophysiology (LNP) is to advance understanding of the function of the brain as this understanding may be relevant to neuropsychiatric illness. Though several of the different NIH institutes have laboratories of neurophysiology, the research in each of these laboratories has a different emphasis. Within the NIMH LNP the special emphasis is on understanding those brain processes that are pertinent to behavior (both normal and abnormal) and to neuropsychiatric illness. LNP research workers pursue these broad goals using a number of different techniques. Indeed, within the past five years the sorts of techniques that are in use in the laboratory have undergone a dramatic change. A decade ago most of the work in the LNP utilized electrophysiological techniques, but the work being carried out currently depends very heavily on the neuroanatomical and neurobiological techniques that allow discovery of the correlates between brain structure, brain chemistry, and brain function. In order to carry out research involving these new approaches it has been necessary to recruit scientists with a variety of new skills. LNP has been extremely successful in its program to bring in scientists with the necessary expertise, and of the ten positions that the laboratory devotes to scientific personnel, six are utilized for neuroanatomists. There are currently two tenured scientists in the laboratory, one (Evarts) being a physiologist and the other (Herkenham) being an anatomist. It is planned that a third scientist, currently a Staff Fellow, will become tenured. This is Steven Wise, who is an anatomist. Each of these three scientists, (Herkenham, Wise and Evarts) is responsible for one of the three major research programs of LNP.

Herkenham is responsible for a research program dealing with studies in which brain chemistry, receptor localization, brain metabolic activity, and brain development are correlated with information obtained from fiber tracing techniques using autoradiography and horseradish peroxidase. The techniques that Herkenham and his colleagues have worked out hold great promise for providing new insights into possible abnormalities of neurotransmitter receptors in a variety of neuropsychiatric illnesses.

Wise and the scientific staff members in his group are carrying out a series of projects aimed at understanding the way in which the different regions of the cerebral cortex interact in the control of movement. Whereas Herkenham and his group emphasize receptor binding techniques and fiber tract tracing, the work of Wise and his group is concerned with neurophysiological correlates of behavior. The studies of Wise combine anatomical techniques with recordings of brain activity during movement in order to obtain information that correlates the exact region in which a neuron is located with the function that this neuron has.

The third major area of work in the Laboratory of Neurophysiology is carried out by Evarts and his group. This third area may be referred to as Motor Psychophysics and involves studies of brain activity in subhuman primates and, in addaddition, studies of motor function in normal subjects and patients with neuropsychiatric disorders. Studies in subhuman primates are aimed at discovering how movements are programmed by the brain, with an emphasis on furthering our understanding of the ways in which sensory information is utilized in controlling

movement. A key technique in studies of the subhuman primates is the recording of activity of individual nerve cells in many different parts of the brain. Such recording has no clinical justification in human subjects but it is possible to record muscle activity and a number of indices of movement in human subjects and by combining the information obtained from the subhuman primates with that obtained from man it is possible to make reasonable inferences as to the processes occurring in the brains of human subjects during motor performance. The purpose of this combined examination of motor control in man and monkey is to obtain a better understanding of how the brain controls movement and how disease of the brain interferes with motor control in neuropsychiatric illness.

In what follows there will be a summary of the work in each of these three major divisions of the LNP research program.

I. Functional Neuroanatomy:

Work in this area is guided by Miles Herkenham and carried out in collaboration with Sandra Moon Edley and Ronald Hammer. The projects now in progress may be divided into two groups, one involving neurochemical approaches to neuroanatomy and the other dealing with metabolic correlates of brain activity. In the summary that follows these two approaches to "dynamic brain structure" will be dealt with in two parts.

Neurochemical Investigations:

For the past four years, a collaborative effort with Dr. Candace Pert in the Biological Psychiatry Branch of the NIMH has been directed toward localizing the brain sites of action of pharmacologically active drugs and putative neurotransmitters. The discovery by Dr. Pert and others in 1973 of brain receptors for opiates and opioid peptides, and the subsequent localization of these receptors by neuroanatomical techniques, has opened up an important new area of anatomy. The task of finding the neuronal circuitry that is "plugged into" these receptor sites requires knowing precisely both the distribution of the receptors and terminal distributions of fiber pathways in any given region. By such analysis one can identify the specific neuronal systems that contain the relevant transmitters and thereby make predictions about the neurochemical's role in normal brain function. Determinations of the locations and densities of the receptors in relevant brain regions can be used in tests of receptor changes during development, after chronic pharmacological manipulation, or in neuropathological tissues.

Using these approaches, Herkenham and his associates have successfully developed an *in vitro* autoradiographic technique for visualizing drug and neurotransmitter receptors in slide-mounted tissue slices. The developed film autoradiogram can be analyzed by a densitometer for computer-assisted quantification of receptor densities.

Results from autoradiography and biochemistry together have supported the hypothesis that the two major opiate receptor subtypes are different conformations of a single, interconvertible, dynamic receptor. In a similar fashion, the phencyclidine receptor was characterized and visualized. Visualization of opiate receptor ontogeny, both pre- and postnatally, suggested other dynamic aspects of opiate receptors: they appear very early in development and, through the process of timed birth and selective elimination, are sculpted into the adult pattern.

Such changes may reflect an important role that receptors play in the establishment of neuronal connections. Opiate receptors undergo phylogenetic changes as well, as suggested by our finding that the ratio of μ to- δ receptor binding increases in parallel with "relatedness to humans" (as indicated by cytochrome C analysis of several vertebrate species). Analysis of μ opiate receptor distribution in rhesus monkey cerebral cortex has revealed several general principles; e.g., opiate receptor density is greatest in limbic and polysensory (association) cortices.

These findings, taken together, suggest a role for opiates in brain function that is much more complex than previously thought and indicate that further analysis of the dynamic aspects of the receptor, after pharmacological or behavioral manipula lations, might enhance our understanding of its function. Ultimately, we might hope to determine the role that opiates and related neurochemicals play in human brain function, especially in receptor-mediated mental disorders or neuropathology.

Metabolic Correlates of Functional Activity:

Taking advantage of the proximity of Sokoloff and his expertise in use of deoxyglucose to study brain metabolism, we are currently involved in developing high-resolution autoradiographic techniques for the cellular localization of metabolic activity at the light microscopic level. Patterns of metabolic activity marked by [^{3}H]2-deoxyglucose uptake are compared in normal, alert rats and in animals given drugs or prior behavioral experience. Using series of adjacent tissue sections from a single animal, patterns of metabolic activity during drug administration can be correlated with the localization of receptors to which the drug binds.

Visualization of metabolic activity of individual neurons requires fixation of the diffusable 2-DG molecule to its uptake site. This may be accomplished by the fixation of 2-DG *in situ* using perfusion with a light-to-medium strength paraformaldehyde fixative followed by cryosectioning and mounting. After perfusion, 2-DG and its labeled metabolites are retained in brain tissue. Moreover, labeling of cellular regions is enhanced. Current evidence suggests that some of the 2-DG may be incorporated into intracellular glycogen, which is fixed in place during perfusion and does not diffuse from its cytoplasmic location during later phases of aqueous processing. Alternatively, these sections can be quickly dried and exposed to tritium-sensitive film to retain in place the labeled diffusable 2-DG-6-phosphate, which is the major 2-DG breakdown product.

Autoradiographic localization of brain receptors can be compared in the same animal with manipulation-induced alterations in brain metabolism measured by the 2-DG technique. Alternate sections from an animal previously injected with 2-DG are either processed for 2-DG autoradiography as described or for receptor localization. The latter is accomplished by first removing the diffusable 2-DG in preincubation solutions prior to *in vitro* receptor binding. In this way, the alteration of brain metabolism by drugs or anesthetics may be correlated with receptor binding in those brain regions affected.

Analysis of 2-DG uptake in animals given anesthetic doses of ketamine has confirmed that phencyclidine-induced changes in brain metabolism occur primarily in limbic regions. Another striking variation is the relatively greater 2-DG uptake in neocortical columns, many of which are in register with granule cell-

poor zones of layer IV in somatic sensory-motor cortex. The columnar patterns of uptake occur in restricted cortical zones innervated by nonspecific thalamic or intracortical fibers. The significance of this relationship of selective cortical afferent innervation and its differential activation during ketamine anesthesia is currently under investigation. Superficial layers of the entorhinal cortex and subiculum also show selective and dramatic increases in 2-DG uptake. Assuming that ketamine's effects on metabolism are initially mediated by its action at the phencyclidine receptor, such increased activity may be correlated with the patterns of tract terminations originating in regions rich in these receptors. These results suggest that phencyclidine influences brain metabolism by selectively affecting the activity within neo- and paleocortical systems.

II. Motor Psychophysics:

This second major LNP research area is directed by Evarts and involves collaboration with a number of investigators in NINCDS. Work carried on in this project has two facets. The first involves studies of motor function in patients with neuropsychiatric disorders as well as in healthy control subjects and is aimed at understanding the basis for motor abnormalities in neuropsychiatric illness. The second involves study of activity of nerve cells in monkey cortical and subcortical structures. The activity of these nerve cells is observed in association with voluntary movement, and the data obtained provide information as to the mechanisms underlying control of movement by the brain.

Human Studies:

The first approach records muscle activity and kinematics of limb position while (1) subjects manually match a target display with either a skilled rapid or slow movement with a handle whose displacement controls a visual display or (2) maintain postures when limb position is passively changed. Movement amplitude, presence or absence of visual feedback of position, disturbances of the subject's movements and changes in sensory input are independent variables. The second approach records a variety of psychomotor variables from patients with Parkinson's disease. The relationships between movement speed, movement accuracy, target size and movement amplitude were studied to develop sensitive measures of psychomotor performance that correlated with clinically determined fluctuations in drug efficacy.

The importance of afferent information in motor control is readily apparent in patients with neurological disorders such as peripheral neuropathy resulting in sensory loss, but tests of motor skills have not yet developed to the point of providing us with useful quantitative indices of the way in which afferent input is used in controlling motor output. Use of physiological techniques combined with motor psychophysics and modern computer displays, together with collaborative work with clinical neurologists, is allowing the present project to clarify a number of longstanding problems of sensory control of movement. Thus, it has been found that the postural responses of patients with cerebellar ataxia and sensory neuropathy are different from normal subjects in that there is a greater variability in the static responses for all sizes of passive displacement. Furthermore, the end point error of movements by patients with sensory neuropathy is extremely sensitive to preloads.

Another procedure to determine motor acuity of normal subjects and patients with neurological diseases involves determining the spatial accuracy of repetitive step movements of various sizes. Both normal subjects and patients with peripheral sensory neuropathy performed large and small movements accurately when visual guidance was available. Movement accuracy deteriorated for both normals and patients when visual guidance was absent, but the patient with sensory neuropathy showed two to four times more error than normal subjects. Furthermore, whereas the error of normal subjects decreased in absolute magnitude when smaller movements were performed, the spatial error exhibited by deafferented patients decreased only a small amount when the smallest movements were performed.

A second facet of work in human subjects has involved development of a computerized system for obtaining objective and quantitative measures of motor function in patients with neurological disorders. Following the partial completion of the system in the summer of 1979, the measurement apparatus was installed in an appropriate setting for use in testing patients with neurological disorders in the Clinical Center. The system has been used to record and subsequently summarize data concerning motor functions in more than 50 patients with Parkinsonism participating in clinical trials of the experimental anti-parkinsonian drugs, lisuride, bromocriptine and pergolide. Patients with cerebellar disorders and essential tremor have also been studied. To establish normative data for movement variables measured on our system, thirty age-matched normal subjects have also been tested. An additional use of the computer system has been to begin studies of psychomotor performance using the methods devised by experimental psychologists. These studies will be useful to provide objective measures of the organization of motor performance and to assist the clinician in diagnosis and evaluation of patients with sensorimotor disorders.

Studies of age-matched normals revealed a considerable range of responses in the parameters measured by the system, with values varying between individuals as a result of such factors as physical stature, motivation, and temperament. Individual scores, however, tended to be relatively consistent over time. Such expected outcomes as diminished speed of movement among members of the oldest age group when compared to younger normal subjects were observed. Parkinsonian patients undergoing treatment with lisuride, bromocriptine or pergolide were tested on a regular basis throughout the course of the buildup and placebo phases of the clinical trial. Grouped data, according to age, confirmed clinical observations in assessing motor performance in individuals undergoing treatment or evaluation. Immediate evaluation of patient performance is available to the test administrators at the completion of each test and periodic summaries of performance may be prepared easily. Furthermore, data collected in all studies to date has been permanently stored in computer files and is accessible for further study. These features of the system could be valuable for longitudinal studies of patients with neuropsychiatric disorders.

These two aspects of the work in human subjects will provide clarification of how tactile and kinesthetic sensory inputs are used to control skilled motor activity, clarification that is essential to the understanding of normal and abnormal motor behavior in humans. In addition, these studies will potentially develop standards of normal motor function and allow comparisons with patients with motor disorders to evaluate subclinical deficits and the efficacy of pharmacotherapeutic agents. The objective evaluation of neuropsychiatric disorders that we have developed should prove useful in a wide variety of experimental applications that require computer recording and analyses of results. Long-term

evaluation of patient's progress on medication regimens is particularly suited for objective analysis.

Brain Activity During Movement: The second part of work on motor psychophysics involves utilization of single neuron recording and operant conditioning techniques in behaving monkeys to study brain mechanisms underlying voluntary movement. Monkeys are trained to make precise movements of a handle whose movement controls a visual display, and stimuli are delivered via the handle by means of an electronically controlled torque motor in order to determine how sensory feedback is processed. Using these methods we have shown that (1) motor cortex pyramidal tract neurons (PTNs) exhibit intense modulation during precise small movements involving relatively slight changes in amount of muscle activity. (2) A large proportion of the PTNs in primary motor cortex (MI) are engaged in controlling early-recruited motoneurons during finely-graded movements. (3) MI PTNs are strongly modulated with different loads especially in the vicinity of zero load. (4) There are significant differences between large and small PTNs with respect to their load-frequency relations.

These four general findings mean that just as there is a "size principle" in spinal cord motoneurons (MNs) whereby smaller MNs tend to be tonically active at low levels of muscular contraction and larger MNs are active phasically during intense exertion, so too there is a relationship between axonal conduction velocity and tonic versus phasic activity in PTNs: smaller PTNs are tonically active even during absence of any discoverable muscular contraction, whereas many larger PTNs are silent in the absence of overt muscular activity exhibiting discharge only when muscles become active. A second major difference between PTNs and MNs is seen in the fact that a very large proportion of motor cortex PTNs related to a given movement exhibit intense modulation for movements that involve activity of a relatively small fraction of the corresponding MNs. Indeed, just as a very large part of the motor cortex is focused on those muscles which are important in precisely controlled movements, it is also the case that a very large proportion of PTNs within an area of cortex controlling a given movement is focused on that fraction of the motoneuron pool which is early recruited and which is of critical importance in precise, fine control. Here then, one sees both parallels and differences between PTNs and MNs.

The two facets of this Motor Psychophysics project seek to apply results of animal research on central control of voluntary movement to an understanding of normal and abnormal movements in man. Thus far, the project has shown that the laws of reflex action, long known to operate at the level of the spinal cord motoneuron, also operate at the level of the cerebral cortex in the course of volitional movements. Motor cortex neurons are impinged upon by afferent inputs which constitute the incoming limb of a transcortical servo loop. Thus, the phylogenetically new motor cortex of the mammal is subject to the same laws of reflex action that characterize phylogenetically older components of motor control systems. But in addition to being driven by a servo system which stabilizes movement and posture, motor cortex can be driven by a second major set of inputs, and it is this second set of inputs that underlies internally generated motor programs. These programs, reaching the motor cortex from the thalamus, are themselves a product of activity in red nucleus, basal ganglia, and cerebellum. It is at these sites in basal ganglia and cerebellum that errors of information transfer are especially likely to take place, and knowledge of the errors may help in designing better treatment.

III. Information Processing in Sensorimotor Cortex:

The third major area of research within the Laboratory of Neurophysiology is being carried out by Steven Wise and several colleagues.

One of the major goals of work being carried out by Wise and his colleagues has been to obtain an understanding of the successive transformations of information leading from sensory input to motor output. Such transformations must occur, for example, when a visual stimulus located at a particular point in space is the target for a visually guided limb movement. The visual information must be used to select patterns of muscle discharge that will guide the limb towards the visual target. It seems likely that the transformations involved between visual input and motor output involve passage of nerve impulses between premotor cortex and motor cortex as well as between these cortical areas and basal ganglia. Wise has worked out paradigms which enable him to follow the changes in discharge patterns of neurons in the cortical areas in question.

The work of Wise and his group on cortical information processing deals with

1) Hierarchical Organization in Motor Control Systems
2) Input-Output Coupling in Sensorimotor Cortex
3) Neurobiology of Cortical Cells.

The work in these three areas involves three different research approaches: 1) The work on Hierarchical Organization has a neuropsychological orientation that is designed to provide information as to the way in which frontal lobe nerve cells control complex, highly skilled voluntary movements. 2) The studies of InputOutput Coupling combine neurophysiological and anatomical techniques to identify the cell types that carry out different sorts of information processing in sensory and motor areas. 3) The work in Neurobiology seeks to apply sophisticated electroanatomical techniques (intracellular injections of dyes into antidromically identified cells in studies that at the same time provide information as to the behavioral and pharmacological properties of the cells. In the following summary, each of these three approaches will be considered.

Hierarchical Organization in Motor Control: The general objectives of this project are (1) an improved understanding of the evolution, organization, and role in the control of voluntary behavior of the entire motor cortex, a region which is likely to include, in addition to its "core," the MI cortex, a surrounding neocortical "belt" containing two or more representations of the motor periphery and (2) a better understanding of the cortical fields involved in the sensory guidance of movements and the linkage between sensory signals and motor behavior.

One aspect of this project dealt with premotor cortex and in this work monkeys were conditioned to align two spots of light on a tangent screen in front of the M. One of these spots is controlled by the computer (target), the other by arm movements of the animal (position). The monkey was required to align the spots within a small accuracy "window." After a short period of time the target light jumped to one of six locations. The monkey had to maintain his arm position unchanged until the target light dimmed, at which point he was required to move (flex or extend his forearm) rapidly and accurately to the target position. In one-sixth of the trials, the computer selected a "no-go" situation in which physically identical stimuli signal the animal to make no movement. This experiment was designed to contrast neuronal activity in MI and premotor cortex.

The results of this study have provided the first clear demonstration of "set-related" (in contrast to movement-related) activity in premotor cortex. It was found that most neurons in premotor cortex field change activity markedly before the onset of a voluntary movement and that a substantial population of neurons change their activity in relation to motor set and/or signals which indicate the location of motor targets. It has also been found that at least one class of premotor cortex units is more clearly related to planned motor activity than the signals which trigger the activity.

Input-Ouput Coupling in Sensorimotor Cortex: This second phase of the work by Wise and his group involves a study of the role of sensory inputs to the cerebral cortex in the control of motor behavior in primates. The first part of the project consists of a comparison of neural activity in the somatic sensory cortex (SI) and the precentral motor cortex (MI). The second part of the project is a detailed examination of the signals which the peripheral receptors are sending to the cortex during perturbation of voluntary movements. Such information should provide important clues concerning the pathway taken by peripheral inputs to MI and its role in the initiation and control of movement.

In comparisons of MI and SI in this project a difference between the two areas was the presence in SI of neurons with a non-muscle-like relation to force and position: a muscle which is more active when the forearm is supinated is always more active when supinating force is applied to an immovable object. In SI, a substantial proportion of cells that are active with supinating force (without movement) became more active with the forearm pronated. The presence of both muscle- and non-muscle-like neurons in SI is consistent with that regions' postulated role in sensory processing. In order to interpret inputs which signal a mixture of position and force information it is necessary to have two inputs which code position and force in different ways. In that way two independent functions of two variables are created. In contrast, the lack of non-muscle-like neurons in MI is consistent with its role in motor control. MI output does not uniquely specify force or position independently. Instead it appears to specify muscle tension, which depends on both force and position.

Neurobiology of Cortical Cells: This third area of Wise's work is designed to examine the input-output organization of neurons in the motor cortex. Rats have been chosen as the primary experimental animal since they are widely used in the pharmacological, immunocytochemical and electronmicroscopic studies with which we wish to correlate our neurobiological and neurobehavioral data. The project has three parts: (1) Neuroanatomical and neurophysiological techniques are being employed to characterize the first motor cortex (MI) in rats and determine the connectional relationships of MI with other neural structures. (2) Single cell recording techniques will be used to record neuronal activity from several classes of projection neurons in the forelimb area of MI cortex in awake, behaving rats. In these experiments, the relationship of the neuronal activity to motor output will be examined for each type of neuron, e.g. corticospinal and corticorubral cells. (3) Intracellular recording is combined with intracellular injection of a tracer, horseradish peroxidase, to visualize the intracortical distribution of neuronal processes belonging to identified projection neurons.

In order to antidromically identify cortical neurons sending their axons to different targets, stimulating electrodes are placed in the pyramidal tract, corticospinal tract, contralateral cortex, and in some cases, in other subcortical targets of MI neurons such as the red nucleus. Each antidromically identified cell is injected with HRP. At the termination of the experiment, the animal is perfused, the tissue is sectioned with a vibratome, and the distribution of tracer within the cell is demonstrated with histochemical techniques. Using these techniques, both pyramidal tract neurons and commissural neurons have been labeled by intracellular injections of HRP. Examination of the axonal arborizations of these cells reveals that both types of MI projection neurons have extensive intracortical connections, suggesting that they have an important role in information processing within MI as well as in sending signals to their projection targets.

One of the major advantages of these new procedures is that they will allow anatomical data on individually identified neurons to be correlated with information on receptor localization and ultimately on brain metabolic activity. Thus, the techniques that Wise and his colleagues are developing are gradually being combined with those of Herkenham and his group. The combined approaches of these two groups hold great promise for major advances in understanding of the structural, neurochemical and electrophysiological mechanisms of behavior.

IV. Outlook:

The foregoing sections of this summary have described three major areas of research within the Laboratory of Neurophysiology. Much of this research is newly undertaken and has involved recently recruited scientists whose skills and training equip them to take maximum advantage of the many promising neuroscience techniques that have developed in the last five years. LNP has evolved from a laboratory that was primarily oriented to electrophysiology ten years ago to one that now combines a number of interdisciplinary neuroscience techniques in studies aimed at understanding brain mechanisms in neuropsychiatric disorders. This evolution is still taking place, and within the past year major progress has occurred in the coordination of research within the laboratory and the formulation of new experiments that focus on brain mechanisms of higher brain function. To promote this formulation Evarts has spent part of the year on a work assignment at the Neurosciences Institute of Rockefeller University, a newly established organization designed to promote innovation in neurobiological research. This work assignment has involved two conferences aimed at identifying the most promising approaches to combined behavioralanatomical-neurophysiological experiments in the future. Based on these conferences, a book by Evarts and Wise has been prepared and it seems likely that the conceptual and experimental approaches developed in this book will have a major impact on research in the neurobiology of higher brain function.

ANNUAL REPORT OF THE ADULT PSYCHIATRY BRANCH
National Institute of Mental Health
October 1, 1981 to September 30, 1982

Richard Jed Wyatt, M.D., Chief

This is the second Annual Report from our laboratory under our new name, the Adult Psychiatry Branch. As our name indicates, our interests span the fields of neuropsychology, neurobiochemistry, neuropharmacology, neuroanatomy and neurophysiology, especially as these disciplines influence research on the schizophrenias and the problems of the aged. As has been said many times, psychiatry must incorporate a number of subspecialties into the investigation of mental illness and mental health. The Adult Psychiatry Branch synthesizes the talents of many physicians and researchers from various specializations, as we attempt to unravel the mysteries of the human brain.

Scientifically, the 1981-1982 reporting period has been an excellent year for us. We have performed some very exciting research, producing significant and interesting results. Our Laboratory's most substantial research concentration is directed towards the schizophrenias, and one of the many pieces of fine work in this area comes from Dr. Janice Stevens. Dr. Stevens, in preliminary work, appears to have histologically isolated evidence of cytomegalovirus in selected autopsied brains of schizophrenic patients. I am looking forward to watching her work develop and reporting her subsequent findings in next year's Annual Report.

Dr. Steven's work, as well as that of many other of our researchers', has been facilitated considerably by Dr. Kleinman's growing collection of autopsied brains. Dr. Kleinman's collection is now over 200 and his autopsy work has resulted in a variety of catecholamine studies. This collection continues to grow and will serve, as it has already, as a substantial resource for our Branch and the Intramural Program.

In our search for potential biochemical markers of the schizophrenic syndrome, Drs. Jeste's and Potkin's work examining phenylethylamine (PEA) excretion continues to produce findings of increased urinary PEA excretion in paranoid schizophrenic patients, employing the sensitive assay developed by Dr. Farouk Karoum. They have substantiated results from a Washington, D.C. study in populations in India, and have begun correlating urinary PEA excretion concentrations with other biochemical parameters. Thus far, the results support our hypothesis that paranoid schizophrenics comprise a physiologically distinct subgroup (i.e., peripheral biochemical parameters such as platelet MAO and urinary PEA excretion, as well as biochemical findings at autopsy, can be used to differentiate paranoid schizophrenics from other psychiatric patients and normal controls).

Drs. Markku Linnoila, William Potter, Farouk Karoum and Steve Potkin have recently demonstrated that some patients with atypical bipolar affective disorders, characterized by psychotic manias and depression, also have fluctuating and episodically high urinary PEA excretion. In treating a 30-year-old woman with this disorder with carbidopa, they found that, although the carbidopa did not affect the duration or severity of the patient's mood cycles, this treatment stabalized her urinary PEA output at a low normal level.

In other biochemical research, Drs. Steven Zalcman, Leonard Neckers, now at the National Cancer Institute, NIH, and Oguz Kaayalp, using the muscarinic cholinergic ligand (^{3}H)-quinuclidinyl benzilate, demonstrated that intact, viable human lymphocytes possess specific muscarinic binding sites. The binding is saturable, proportional to cell number, and is displaceable by atropine, benztropine, trihexyphenidyl and scopolamine. We feel that not

only do these findings provide a pharmacological basis for the observed effects of muscarinic agents on lynphocyte function, but they also demonstrate the utility of human peripheral blood lymphocytes for investigation of abnormalities in the muscarinic cholinergic system.

While we report our sleep study research in the Annual Report of the Biological Psychiatry Branch on the Bethesda campus, I would like to mention here Drs. Gillin's and Mendelson's work showing a decrease in glucose utilization during sleep in monkeys. Also, Dr. Mendelson has found that 3-hydroxymethyl-Beta-carboline, which binds to the benzodiazepine receptor and has been reported to antagonize several of the actions of benzodiazepines, induces a dose-dependent increase in sleep latency in rats. At a low dose, too low to affect sleep, 3-hydroxymethyl-Beta-carboline blocks the sleep-induction produced by a large dose of flurazepam. Dr. Mendelson's results suggest that the benzodiazepine receptor may play a role in sleep regulations as well as pharmacologic sleep induction.

In the area of clinical assessments, two particularly innovative directions have been developed. Ms. Lannie Spoor and Dr. Steven Potkin have designed the Premorbid Adjustment Scale (PAS). The PAS addresses the problems of conceptualizing premorbid adjustment concerning the attainment of certain developmental goals (those viewed most frequently as necessary milestones for healthy functioning) and the attainment of those goals as specific age-related tasks. It was created to evaluate, at each of several periods of the subject's life, the level of functioning in four major areas: social accessability versus isolation, peer relationships, ability to function outside the nuclear family, and the capacity to form intimate socio-sexual bonds. The scale is intended to measure only premorbid functioning and after initial reliability and validity trials on various healthy and ill populations, the PAS appears to be a potentially major contribution to our body of assessment tools .

The second assessment innovation is the creation of Dr. Lewellyn Bigelow. Together with Mr. Ivan Waldman, they have developed a computer program to allow online entry of nursing behavioral data directly from our three inpatient units. This program enables the rater to respond to serially presented questions regarding each patient, as well as permitting rapid correlations of behavioral, physiological and biochemical data.

Over the past reporting year, our work employing computerized axial tomography (CT) scans has grown. Building on Dr. Daniel Weinberger's previous findings of enlarged cerebral ventricles in the brains of some schizophrenic patients, we have been investigating possible reasons why such enlargements occur. Through several series of studies, we found that the patients with CT abnormalities had significantly poorer adjustment during childhood (ages 5-15) than did the controls. We feel this suggests that either the atrophic (or lack of normal brain development) changes themselves, or the processes ultimately responsible for them, are early developmental phenomena and not an acute event in a previously well functioning individual. Further support for the notion that CT abnormalities precede the onset of schizophrenia in adult patients comes from Dr. Lynn Delisi's finding that enlarged ventricles occur in first break schizophrenoform patients. Also, Dr. Weinberger has found no correlation between ventricular size and duration of illness.

Looking for markers for schizophrenia from another perspective, Dr. John Morihisha, together with Dr. Frank Duffy at Harvard Univeristy, has recently introduced the technique of brain electrical activity mapping (BEAM) to the study of schizophrenic patients. They have begun applying BEAM, previously applied to such neurological problems as brain tumors or functional deficit dyslexia, to this disorder and have found that there is an increased amount of frontal delta wave activity in eleven unmedicated chronic schizophrenic patients.

Finally, together with Dr. Monte Buchsbaum, we are beginning studies using positron emission tomography (PET) to elucidate the metabolism of neuroleptic drugs. By studying changes in local glucose metabolism in schizophrenic patients on and off neuroleptics, and normal control comparisons, we hope to provide new information on the pathophysiology of schizophrenia and on drug action.

Investigating metabolic processes in another vein, Dr. Freed performed a series of studies to examine the effects of calcitonin on the eating patterns and appetites of rats. Of the little known about the effects of calcitonin, it has been postulated that this hormone has anorectic properties. Dr. Freed's results confirm this hypothesis. The rats were given single subcutaneous injections of synthetic salmon calcitonin, and their eating, drinking, urine and fecal excretion, and weights were measured for the following 24 hours. Calcitonin produced a dose-dependent inhibition of eating and a concommitant decrease in fecal excretion and body weight. All of these effects recovered over the succeeding 24 hours.

Although we are phasing out most of our drug abuse research, our work, with the collaboration of Dr. Betsy Parker, NIAAA, has continued in the area of alcohol use. Dr. Parker's work this past year, focused on alcohol related cognitive deficits, has begun to deliniate among the various types of cognitive processes affected by alcohol consumption. For example, Dr. Parker found that intoxicated subjects could not utilize normally effective but subtle memory cues and, although self-generated reminders improved recall slightly, they did not eliminate or reduce the negative effects of the alcohol. Interestingly, assessment of the self-generated cues showed that intoxicated subjects produced qualitatively similar reminders to those of sober subjects. In another series of studies, Dr. Parker and her co-workers found that although intoxicated subjects appear capable of making simple judgements based on previously familiar information, they exhibit substantial deficits when asked to recall newly learned information.

In our Aging Program, our work exploring Alzheimer's disease has progressed over the past year. Dr. David Shore's work investigating the significance of the relationship between aluminum accumulations and Alzheimer's disease confirms that the increases in nerve cell nucleus aluminum, that have been previously reported, are not the result of a generalized overload of this metal in biological fluids. Dr. Shore has begun a therapeutic study of Alzheimer's patients to study whether or not the progressive deterioration of this disease can be slowed by oral administration of sodium flouride. In this longitudinal study, he is also investigating the degree and type of language impairment in Alzheimer's disease, the use of memory, and language tests. In collaboration with Dr. Daniel Weinberger, Dr. Shore is examining the CT scans of Alzheimer's patients, and with Linda Nee, MSW, he is trying to determine whether there is a correlation between autosomal dominant inheritance of Alzheimer's disease and the ethnic background of the families. Finally, Dr. Shore has been working to develop a test of lymphocyte vulnerability to aluminum toxicity.

In another series of studies within our Aging Program, Drs. Peter Bridge, Bruce Phelps, Neal Cutler, and Dilip Jeste examined dopamine-beta-hydroxylase (DBH) and monoamine oxidase (MAO) activity in a group of elderly schizophrenic patients with respect to cognitive functioning. (DBH and MAO are two of the enzymes important in norepinephrine (NE) metabolism). They found that the demented patients had lower platelet MAO values than either demented schizophrenic or nondemented controls and that the schizophrenic patients and the controls revealed divergent patterns of plasma DBH activity across demented/nondemented status.

Dr. Luis de Medinacelli, in work in a different direction, is attempting to develop a nerve repair technique. Dr. de Medinacelli's work with rabbits is most promising and I look forward to reporting significant progress in his work in next year's Annual Report.

Our brain grafting research, also, has been moving steadily forward. Given the success of our experiments grafting fetal rat substantia nigra into the denervated caudate nucleus of older rats, we sought other, non-fetal tissue that might be suitable for transplantation. To this end, Dr. William Freed and Ms. Lannie Spoor have transplanted adult adrenal medulla cells into host caudate and have met with success again. We are now experimenting with rhesus monkeys, as we continue to observe the rat transplants. Intriquingly, for both the fetal substantia nigra and adult adrenal medulla grafts, after almost a year since transplantation and unlike the rest of the animals' brains, the grafts show no substantial signs of aging. Further, to examine the grafted tissue more rigorously, Dr. Alan Fine, before he left the Branch for the Weitzman Institue in Israel, developed a new staining procedure that allows us to more clearly see the transplanted tissue histologically. This new staining procedure takes advantage of a shift in catecholamine fluorescence excitation, producing a simple but stringent test for specificity.

Related to the grafting work, Dr. Freed performed a series of studies grafting entire eyes from fetal rats into the brains of blind adults. In most cases, grafts placed deep within the brain grew and differentiated, and were found to contain retinal cells when examined histologically. The grafts were found to be sensitive to light, in that electrical potentials similar to electroretinograms (ERGs) or partial ERGs were evoked by flashes of light.

In a study of the cellular basis of kindling, (kindling is the progressive lowering of seizure thresholds with repeated stimulation), Adolphus Oliver and Dr. Barry Hoffer of the University of Colorado Health Sciences Center, Denver, studied slices of guinea pig hippocampus electrophysiologically. Normally, epileptiform activity can be induced in slices only by combined exposure to elevated potassium levels and a chemical convulsant such as penicillin. Mr. Oliver and Dr. Hoffer have shown, however, that in hippocampal slices from pentylenetetrazole-kindled animals, elevated potassium alone can induce seizures. These data suggest that kindling elicits long-term changes in neuronal excitability that may involve ionic mechanisms.

The other major direction of research in our Aging Program concerns the neuroleptic treatment complication of tardive dyskinesia (TD). Because of the alarming rise in the incidence of this motor dsyfunction (our epidemeological survey indicates that about 25 percent of patients given neuroleptics, chronically, are likely to contract TD), Drs. Dilip Jeste and Richard Wagner are studying this condition from several directions. Among their findings to date, they have determined that in older patients i)tardive dyskinesia is significantly correlated with high blood serum concentrations of neuroleptic drugs, ii) no significant correlation exists between TD and obvious structural brain abnormalities and iii) those most likely to develop tardive dyskinesia have low platelet MAO activity and high serum dopamine-B-hydroxylase activity.

Our scientific achievements are the result of the cumulative efforts of an unusually talented group of scientists, some of whom have been with us for several years and others who are new to the laboratory. For those who are newly arrived we would like to take a moment to officially welcome them, as we say good-bye to others. This past year we have been joined by Drs. Scherer, Kirch, Iager, Putnam, Esa Korpi, and Joe Martin and look forward to their contributions to the Branch. Also, we welcome Drs. Wolf and Mosnaim for the summer, on leave from their posts at the University of Chicago. We have been keeping in touch with Dr. Steve Potkin at the Beijing Medical Center in Peking and look forward to his return. Nor would our welcome be complete without mentioning Larry Ray and Bob Lilly. Having joined us as a result of the reduction in force (RIF) process, they have made the best of a bad situation and have become integrated members of the Laboratory.

Those we have said good-bye to are Drs. Lynn DeLisi, who has joined Dr. Monte Buchsbaum's group in Bethesda, Alan Fine, Steve Zaleman, who is working with grants at the NIH, Chris Gillin, who has moved to a professorship at the University of California at San Diego, Andrew Sostek, Dave Reiss, and Neal Cutler, who is now the Chief of the new Intramural Program, Clinical Studies Section, at the National Institute on Aging. We wish them all much success in their new positions. Finally, as I look back over the past year, I view it as one of high scientific achievement, energy and exciting progress for the Adult Psychiatry Branch. I look forward to reporting the same, this time next year.

ANNUAL REPORT FY 1982

LABORATORY OF PRECLINICAL PHARMACOLOGY

Erminio Costa, M.D., Chief

The greatest achievement in neuroscience in the last 20 years has been the establishment of ground rules explaining the similarities in the mechanisms directing various neurons to generate, transmit, and receive electrical signals. The biochemical basis for this uniformity was expressed by the concept known as Dale's Law: each neuron uses only one neurochemical transmitter to communicate with contiguous neurons. Hence, if electrical and biochemical signals that instruct neurons and activate neuronal circuits are similar, what is the substrate for the different plasticities and functional capacities in brains of the same species? One possible explanation for these differences is emerging from histochemical studies and is corroborated by neurochemical analysis showing coexistence in the same neuron of two or more putative neuromodulators. To say the least, this finding suggests that the mode in which a neuron instructs the next neuron is not as simple as was proposed by Dale's Law. The study of this new complication in the biochemistry of synaptic transmission, both at the level of the signal generation and at the level of the signal reception, is the primary focus of our laboratory. We presently believe that by decoding these complexities we may discover why the brain is not as predictable as a computer in its functional output and why each brain has a certain degree of uniqueness.

We are studying the consequence of the coexistence of more than one modulator in the same axons using the model of the recognition site, the coupler system, and the transducer. The transducer can transform the chemical signal into a specific ion flux (ion gate opening) or into an enzyme activation (second messenger formation), the gain at which the transducer operates can be set by the operation of the coupler system which receives instructions from the recognition site of the primary transmitter. The informational structure of this outside signal is primary and, therefore, necessary for the opening of the gate and the electrical signal generation. There are, however, other signals that are generated by the same source and instruct the same receptor systems and give quality, plasticity, and creativity to the signal being generated. Hence, contrary to traditional belief that the information processed by a neuron arrives as a set of signals with specified modalities, the coexistence of two modulators creates the possibility that primary transmitter input is specified but that the associated cotransmitter input is elaborated in a variable way by rules that we do not entirely understand.

During this year, we studied at least three different types of models where a plurality of neuromodulators converge to modulate neuronal input elaboration. One model is formed by GABAergic synapses where the recognition sites for benzodiazepines and GABA modulate the function of the transducer (Cl^- gate), using GABA-modulin, a protein which can be phosphorylated as an intermediary coupler.

Another model is represented by the adrenal medulla where from splanchnic nerve and from blood, modulatory influences mediated by opioid recognition sites converge to modulate the acetylcholine receptor.

A third model is given by the antidepressants which act on the recognition sites located in contiguity with the 5HT receptors. Repeated antidepressant injections down regulate the so-called $5HT_2$ recognition site which acts on primary recognition sites for 5HT. This effect is, however, indirect and in the case of imipramine, derives from a modulatory influence of the antidepressants on the uptake of 5HT. Interestingly enough, this uptake, which participates in 5HT receptor function by modulating 5HT recognition site occupancy, is ultimately modulated by an endogenous effector.

A final model of transmitter cotransmitter interaction can be found in the noninnervated pituitary receptors for dopamine and for the vasoactive intestinal peptide (VIP). It is important to note that dopamine function, by modulating VIP, elicited the activation of adenylate cyclase and prolactin secretion. Hence, in this case, dopamine would function as a cotransmitter for VIP.

Section on Neuroendocrinology (A. Guidotti, M.D., Chief)

During FY 1982, the program of this section has focussed on three major research projects: 1) the modulatory role of opiate receptors in the function of nicotinic receptors located in the adrenal medulla; 2) transmitter interaction in the regulation of pituitary function; and 3) molecular pharmacology of GABA receptors.

1. Opiate peptides as cotransmitters of acetylcholine (ACh)

This project was prompted by the need to elucidate the function of enkephalin-like peptides which coexist with ACh in splanchnic terminals. Primary culture of chromaffin cells from bovine adrenal medulla was the ideal model for these studies because these cells are innervated by the splanchnic nerve and possess high affinity stereospecific binding sites for opiates and cholinergic nicotinic receptor agonists, which trigger the release of catecholamines from chromaffin cells.

Dr. Saiani (Visiting Fellow from Italy) has characterized the opiate recognition sites of these cells by using specific ligands for μ, δ, κ and σ receptors. She measured the KD and Bmax of the compounds binding to adrenal medulla membranes and the compound's potency to inhibit the release of catecholamines from chromaffin cells elicited by stimulation of nicotinic receptors. She found that etorphin, beta-endorphin, met-enkephalin-Arg^6-Phe^7 and the synthetic peptide (D-Ala^2-Met-Phe^4-Met (0)-ol-enkephalin) inhibited the acetylcholine-induced release of catecholamines with an IC_{30} varying from 10^{-7} to $1x10^{-6}$M. The effect was stereospecific because levorphanol was approximately two orders of magitude more potent than dextrorphan. Morphine (μ receptor agonist), D-Ala^2-D-Leu^5-enkephalin (δ receptor agonist), ethylketazocine (K receptor agonist) and N-allylnormetazocine (σ receptor agonist) were at least 100-1000 times less potent than etorphin. Diprenorphine and naloxone antagonized the etorphin inhibition of the ACh-induced release of catecholamines in a manner consistent with the view that ACh receptors interact physiologically with opiate receptors. In fact, high affinity, saturable and stereospecific binding sites for ^{3}H-etorphin, ^{3}H-dihydromorphine, ^{3}H-[D-Ala^2-D-Leu^5]-enkephalin, ^{3}H-ethylketazocine and ^{3}H-N-allylnormetazocine, ^{3}H-diprenorphine and ^{3}H-naloxone are located in chromaffin cell membranes and in membranes obtained from adrenal medulla homogenates. However, the number of binding sites for ^{3}H-etorphin and for ^{3}H-diprenorphine was 10 to 70 times higher than the number of sites for the other ^{3}H-ligands combined.

The ranking order of potency of these compounds to displace ^{3}H-etorphin binding correlates with their potency to inhibit ACh-induced release of catecholamines from chromaffin cells. These data suggest that a stereoselective opiate receptor (different from the classical $\mu, \delta, \kappa, \sigma$ receptor) with high affinity for etorphin, diprenorphin, beta-endorphin and Met^{5}-enkephalin-Arg^{6}-Phe^{7} modulates the function of the nicotinic receptor in adrenal chromaffin cells.

Dr. Panula (Visiting Fellow from Finland) found that Met^{5}-enkephalin-Arg^{6}-Phe^{7} is present in splanchnic nerve. It is possible to consider this peptide as the endogenous effector of the opiate receptor present in chromaffin cells.

In collaboration with Dr. I. Hanbauer (Heart, Lung and Blood Inst., NIH), using dogs with cannulated adrenal vein, we have shown that blockade of opiate receptors with diprenorphin increases the amount of catecholamines released by nerve impulses. Currently, we are investigating whether thiorphane, a specific inhibitor of enkephalinase, and captopryl, a specific inhibitor of Met^{5}-enkephalin-Arg^{6}-Phe^{7} can inhibit the release of catecholamines by nerve stimulation. The results of these experiments may reveal a physiological role for the ACh opiate receptor interaction and elucidate whether this interaction can be considered a regulatory site for the development of new antihypertensive drugs. From the data available, it can be inferred that when the Met-enkephalin-like material is coreleased from the splanchnic with ACh, it modulates the action of ACh to release catecholamines from chromaffin cells. In addition, our data point out that adrenal nicotinic receptors are susceptible to a modulation by blood borne beta-endorphin, secreted from the pituitary. Hence, these data suggest an interaction between pituitary and medulla via a beta-endorphin modulation of the catecholamine release from medulla. The molecular mechanisms whereby cotransmitters and ACh interact is currently being investigated.

2. Pituitary regulation by transmitter interactions

In collaboration with Dr. Grandison (Department of Physiology and Biophysics, Rutgers Medical School, Piscataway, N.J.), we attempted to clarify the role of GABA receptors in the control of prolactin release. In fact, several lines of evidence suggest that gamma-aminobutyric acid (GABA), a potent inhibitory neurotransmitter, is one of the endogenous factors controlling prolactin secretion. The widespread distribution of GABAergic junctions throughout the brain and hypothalamus suggests that GABA could act at many sites to regulate prolactin release. However, the recently described tuberoinfundibular GABAergic neuronal system can influence the secretion of prolactin at its final step. The release from mammotrophs of the anterior pituitary may be the most important GABA effector site for the regulation of prolactin secretion. By analogy with the A_{12} dopaminergic neurons that release dopamine in the portal circulation of the pituitary, the tuberoinfundibular GABAergic neurons may release GABA into the hypophyseal portal vessels from which it is carried to the anterior pituitary.

Since evidence implicating a role for GABA recognition sites of pituitary in the regulation of prolactin secretion has been obtained mainly from rodents, before examining this model in humans, we considered it necessary to investigate the presence and characteristics of GABA receptors in human anterior pituitary tissue. The results indicate that human anterior pituitary has GABA$_B$ receptors with a Bmax and KD similar to those reported for the high affinity ^{3}H-GABA binding located in brain membranes. In addition, human pituitary contains considerable amounts of GABA($\sim$ 2.5 nmol/mg protein) and significant amounts of benzo-

diazepam binding sites, but we could not detect any GAD activity.

To investigate the role of GABA in the secretion of prolactin, new tools are needed to stimulate the GABA receptors. With Dr. Forchetti (Guest Worker from Italy) and Dr. F. Moroni (Department of Pharmacology, University of Florence, Italy), we have established that while muscimol is rapidly metabolized by transamination, another GABA analogue, THIP, is not degraded by transamination and, therefore, has a longer lasting biological half-life. The biological profile of THIP suggests that it may be an ideal tool to study the role of pituitary GABA receptors in the secretion of prolactin in man.

3. Molecular pharmacology of GABA receptors

A concerted effort was directed toward the identification, isolation, purification and reconstitution of the various components of the GABA receptor complex. The strategy followed assumed that GABA/benzodiazepine interactions reflect a transmitter/cotransmitter relationship where GABA and benzodiazepine recognition sites interact with each other and with the Cl^- gate when a membrane protein termed GABA-modulin (GM) is present. Perhaps, this GM functions as a coupler device for GABA receptor function.

With Mr. Konkel, Dr. Corda (Guest Worker from Italy), Dr. Ebstein, Dr. Krutzsch (Lab. of Immunogenetics, NIAID, NIH, Bethesda, MD) and Dr. Meek, we have been successful in isolating and purifying to homogeneity GM, a protein of 16,000 MW. This protein inhibits noncompetitively the high affinity binding of ^{3}H-GABA or ^{3}H-muscimol to synaptic membranes and also blocks the stimulation of ^{3}H-diazepam binding elicited by GABA.

Drs. Corda and Ebstein have studied the coupling function of GM in solubilized preparations of the GABA/benzodiazepine receptor; the results indicate that benzodiazepine/GABA recognition site interaction does not occur without GM. Drs. Wise (PRAT Fellow) and Corda have also shown that GM can be phosphorylated in cell free preparations and in slices of brain cortex by a protein kinase. The phosphorylation of GM is dependent on calcium ions and calmodulin. We are currently investigating whether GABA-modulin phosphorylation is facilitated or inhibited by GABA released by nerve stimulation and whether phosphorylation and dephosphorylation of GM participates in the allosteric regulation of GABA/benzodiazepine receptor complex.

We are aware that the role of benzodiazepine recognition sites in brain may be broader than what we had assumed in our working model. In fact, Dr. Shah (Visiting Associate from India) and Dr. Chambon (Guest Worker from France) have demonstrated that benzodiazepines potentiate the binding to brain membranes and the convulsant action of diphenylhydantoin.

The chemical nature and physiological role of the endogenous agonist of the benzodiazepine recognition sites represents a major goal of our laboratory. Dr. Corda's studies have helped to characterize the pharmacological profile of two benzodiazepine antagonists (RO-15-1788 and GGS-8216) and a number of beta-carboline derivatives; this work has suggested that the endogenous effector for the benzodiazepine recognition site may be an anxiogenic and proconvulsant compound; hence, benzodiazepines act as anticonvulsants and anxiolytics act as the antagonits of the endogenous ligand of benzodiazepine recognition sites. Dr. Corda studied the effect of repeated injections of RO-15-1788 and GGS-8216 on the

benzodiazepine recognition site. It is generally accepted that repeated daily injections of agonists cause a down regulation of receptors while repeated daily injections of the antagonist increase the Bmax of the recognition site. However, repeated injections of flunitrazepam or GGS 8216 failed to change the binding characteristics of ^{3}H-diazepam or ^{3}H-beta-carbolines. Hence, compounds that act on the benzodiazepine recognition site by opposite mechanisms fail to change receptor characteristics when injected daily for three weeks. These negative results support the view that the endogenous effector of the benzodiazepine recognition site may not be a primary transmitter. Other cotransmitters, such as opiates, when given daily for two or more weeks fail to down regulate the specific recognition sites. This and other indirect lines of evidence support the view that benzodiazepines act as cotransmitters of GABAergic receptors.

The questions then arise whether all GABA receptors are modulated by the endogenous effector of benzodiazepine recognition sites; whether there is only one physiological effect; or for the benzodiazepine recognition sites, whether these sites are connected only with GABA recognition sites. To answer these questions, Drs. Ebstein and Forchetti attempted to isolate and characterize the endogenous effector of the benzodiazepine recognition site. They isolated, purified, and biologically characterized a peptide (DBI) of approximately 10,000 daltons whose carboxy terminus is tyrosine and whose amino terminus is blocked. DBI is present in rat brain in concentrations of 10 to 25 μM but could not be detected in extracts of liver, spleen, or kidney. DBI interacts with benzodiazepine recognition sites with characteristics similar to those of beta-carboline derivatives which are proconvulsant and cause anxiety in man. Injected intraventricularly into the rat, DBI (50 μg) prevent the anticonflict action of diazepam, indicating that in vivo, DBI causes responses antagonistic to those elicited by benzodiazepines. These data suggest that DBI may function as a naturally occurring anxiogenic compound. However, the question whether DBI represents a physiological and relevant endogenous cotransmitter operative in the GABAergic system remains unanswered. Although DBI blocks the increase in diazepam binding induced by GABA, we cannot infer that DBI has a physiological role until we can show its synaptic location, its coexistence, its release from GABAergic terminals, and its action on the Cl^{-} channels regulated by GABA. In addition, the large molecular weight of DBI may suggest that we are isolating a precursor of the endogenous effector of the benzodiazepine recognition site. The physiological effector may have a smaller molecular weight. The rapid degradation of this small molecular weight peptide could not be detected with the present techniques.

Section on Molecular Neurobiology (E. Costa, M.D., Acting Chief)

The work of this section can be separated into three groups, headed by Drs. Yang, Schwartz and Chuang. In the past year, the activity of Dr. Yang's group has been focussed on studies of biosynthesis, degradation and distribution of two endogenous opioid peptides, Met^{5}-enkephalin and Met^{5}-enkephalin-Arg^{6}-Phe^{7}. Met^{5}-enkephalin and Leu^{5}-enkephalin are derived from the 27.3 K daltons of proenkephalin, which contain six sequences of Met^{5}-enkephalin and one sequence each of Leu^{5}-enkephalin and Met^{5}-enkephalin-Arg^{6}-Phe^{7}. Although many fragments of this proenkephalin have been identified, its enzymatic degradation to form enkephalins (penta and heptapeptide) is still unknown.

Dr. I. Lindberg (PRAT Fellow) found that the protein fraction prepared from

bovine adrenal chromaffin granules contains both an enzyme and proenkephalin. Therefore, it is possible to generate Met^5-and Leu^5-enkephalin as well as other yet unidentified low molecular weight enkephalin-like immunoreactive peptides, when this protein fraction is incubated at 37°C. This Met^5-enkephalin generating activity was inhibited by soybean trypsin inhibitor. By taking advantage of this property, the enzyme was successfully separated from its substrate, the proenkephalin, and was partially purified. This enzyme preparation can generate Met^5-enkephalin from endogenous substrates without the participation of carboxypeptidase B. The enzyme was not inhibited by a sulfhydryl reagent, such as p-chloromercuriphenyl sulfonate, nor stimulated by the sulfhydryl reagent, dithiothreitol, suggesting that this enzyme is not cathepsin B of lysosomal origin. The enzyme was found to be maximally active at a neutral pH, further differentiating this enzyme from cathepsin B. The enzyme was inhibited markedly by soybean trypsin inhibitor, aprotinin, and diisopropylfluorophosphate (DFP) but not by N-alpha-Tosyl-L-Lysyl chloromethane (TLCK). These results indicate that this enkephalin generating enzyme is a trypsin-like serine protease, but is not identical to trypsin.

Several lines of evidence have suggested that dipeptidyl carboxypeptidase may play an important role in enkephalin metabolism. In addition to Met^5-and Leu^5-enkephalin, also Met^5-enkephalin-Arg^6-Phe^7 (YGGFMRF) can be hydrolyzed by a dipeptidyl carboxypeptidase. In order to determine whether the dipeptidyl carboxypeptidase participates in the metabolism of enkephalin and YGGFMRF, Dr. Yang studied the effects of various dipeptidyl carboxypeptidase inhibitors on the degradation of these two peptides both in vitro and in vivo.

Ms. Majane and Dr. Yang showed that enkephalin inactivating dipeptidyl carboxypeptidase (enkephalinase) and YGGFMRF converting activity can be selectively inhibited by different inhibitors. Captopril inhibited the YGGFMRF converting activity with an IC_{50} of $5x10^{-8}$M, while it was nearly totally inactive toward enkephalinase. Acetthiorphan, on the other hand, efficiently depressed enkephalinase with an IC_{50} of $3x10^{-7}$M but exerted little effect on YGGFMRF converting activity. A similar differential effect was also observed with another enkephalinase inhibitor, thiorphan.

Drs. An-zhong Zhang (Visiting Fellow from The People's Republic of China) and Dr. Yang found that intracerebrally injected thiorphan increased striatal Met^5-enkephalin content and inhibited the degradation of this brain peptapeptide markedly. The thiorphan injection also elicited a naloxone reversible prolongation of the jump latency from a 54°C hot plate but not from the paw licking latency. This behavioral response can also be mimicked by intracerebral injection of a low dose of Met^5-enkephalin. The results taken together suggest that enkephalinase may play an important role in the physiological inactivation of enkephalin. However, it should be noted that bestatin, an aminopeptidase inhibitor, can potentiate the effect of thiorphan, but shows no effect of its own. The result suggests that aminopeptidase may also participate in the activation of Met^5-enkephalin released extraneuronally. We do not know whether both enzymes are located in this specific synapse. Captopril injected intracerebrally was found to increase the striatal YGGFMRF content to a small degree in mice, but not in rats. The injection of captopril also potentiated the analgesic effect of intraventricularly injected YGGFMRF. Dr. Tang (Visiting Fellow from the Peoples Republic of China) and Dr. Zhang found that in rats injected intracerebrally with captopril and then treated with electroacupuncture, the striatal content of YGGFMRF was doubled but the striatal content of Met^5-enkephalin was

unchanged. This effect was not observed when the rats were treated with either captopril or acupuncture alone. The captopril injection also increased the duration of acupuncture analgesia considerably and this effect was antagonized by naloxone treatment. These results suggest that the captopril-sensitive dipeptidyl carboxypeptidase may be responsible for the physiological inactivation of Met^5-enkephalin-Arg^6-Phe^7. The results further suggest that the specific inhibitors for enkephalin and YGGFMRF inactivation may provide a useful tool to study the physiological role of these two opioid peptides.

In order to explore whether YGGFMRF may also function as a Met^5-enkephalin-precursor, Dr. Tang compared the YGGFMRF distribution in brain and in peripheral tissues with the distribution of Met^5-enkephalin. He found that YGGFMRF immunoreactivity is unevenly distributed throughout the rat brain. The highest content is in the striatum and hypothalamus, and the lowest in the hippocampus and cerebellum. Histochemical studies by Dr. Panula on the brain location of YGGFMRF-like immunoreactive material has revealed a distribution similar to Met^5-enkephalin in most brain structures. In the hippocampus in the perforant pathway, however, YGGFMRF containing axons do not appear to contain Met^5-enkephalin. Also in the hippocampus, the content of YGGFMRF is smaller than that of Met^5-enkephalin. The similar distribution raises the possibility that these two peptides may be localized in the same neuron. A chromatographic analysis of the immunoreactivity performed by Dr. Yang has shown that the YGGFMRF immunoreactive material in striatum and hypothalamus was composed mainly of YGGFMRF. Interestingly, high molecular weight YGGFMRF immunoreactive material (HMW-YGGFMRF) was detected in some brain regions, such as the cortex, medulla, and midbrain. This HMW-YGGFMRF has been partially purified in extracts from medulla and midbrain. Further purification and characterization of this HMW YGGFMRF is now in progress.

The histochemical distribution studies of YGGFMRF in the hippocampus performed by Dr. Panula indicate that this peptide may act as an independent neuromodulator. This possibility is supported by studies of Dr. Tang showing that the heptapeptide can be released from striatal slices by depolarization in a Ca^{2+}-dependent manner. Dr. Panula found that the YGGFMRF is located in terminals of striatum. Drs. Tang and Chou (Guest Worker from the People's Republic of China) found that the heptapeptide is widely distributed in peripheral tissues. Intestine, lung and superior cervical ganglia were found to contain high levels of YGGFMRF. In contrast to brain, the distribution of YGGFMRF does not parallel that of Met^5-enkephalin. Dr. Panula found that in lung, YGGFMRF immunoreactivity is localized in APUD-like cells closely associated with bronchi. The YGGFMRF immunoreactive material can be released when rat lung tissue slices are perfused with 47 mM KCl and this release is Ca^{2+} dependent. Drs. Zhang, Chou, and Tang found a high affinity opiate recognition site in rat lung membrane preparation using ^{3}H-etorphin as an opiate ligand. This receptor also was shown to have a high affinity for YGGFMRF but not for naloxone. These results seem to indicate that YGGFMRF may play an important role in respiratory function. The lung may provide a very useful model to study the physiological role of YGGFMRF separately from Met^5-enkephalin which is not detectable in the lung of rats and guinea pigs. Dr. Tang detected Substance P and bombesin in lung extracts and Dr. Panula found that in contrast to YGGFMRF, these neuropeptides were localized in nerves that innervated the bronchi.

Food consumption appears to be under inhibitory control of serotonergic neurons projecting from the dorsal raphe nucleus to the amygdala and hypothalamus. Evidence suggesting the involvement of endorphins in the regulation of eating behavior is accumulating steadily. In order to explore the possible interaction

between serotonergic and endorphinergic actions, Dr. Harsing (Visiting Associate from Hungary) studied the effect of d-fenfluramine and CM 57-277, two drugs that enhance serotonergic activity, on the hypothalamic content of Met^5-enkephalin and beta-endorphin. Repeated injections of d-fenfluramine and CM 57-277 resulted in an elevation of Met^5-, Leu^5-enkephalin, and beta-endorphin in the hypothalamus but not in other areas. This response was accompanied by a reduction in body weight. The effect of these two anorectics was antagonized by methergoline, a serotonin receptor antagonist, and p-chlorophenylalanine, a serotonin synthesis inhibitor. The hypothalamic content of other neuropeptides, such as cholecystokinin and Substance P was not modified in rats receiving repeated injections of the two anorectic drugs. The results obtained in this study suggest that the accumulation of endorphin reflects a decrease in utilization; perhaps, the release of hypothalamic endorphins is decreased during the increased serotonergic activity elicited by CM 57-277 and d-fenfluramine. Hence, d-fenfluramine's anorectic activity may be due to a lack of enkephalinergic tone in hypothalamus and, therefore, is sharply differentiated from that elicited by d-amphetamine.

Immunocytochemical studies have suggested the coexistence of dopamine (DA) and cholecystokinin (CCK) in neurons of the substantia nigra (SN) ventral tegmental (VTA) complex, projecting to the nucleus accumbens (NA) and olfactory tubercle (OT). Dr. Iadarola (Guest Worker from USA) studied this coexistence using various lesions of the mesencephalic DA system as a tool. After hemitransection, CCK content was reduced to about 60 percent in OT and 50 percent in NA. No change in CCK was detected in caudate putamen (CP) where tyrosine hydroxylase (TH) was reduced to 15-30 percent. After a lesion of the dopaminergic neurons by infusion of 6-hydroxydopamine (6OHDA) into either the SN or VTA complex, the maximal loss of CCK was about 50 percent in both OT and NA. The results appeared to be dependent on both location and extent of the lesion. From data obtained from these, and other lesion studies, a number of provisional conclusions can be made: 1) approximately 50 percent of the CCK content of the NA and OT is not associated with mesolimbic DA neurons; 2) there is an approximate 30 percent distribution to NA and OT of cells lateral to VTA that contain DA but not CCK; 3) nearly 70 percent of the DA innervation of OT and NA contain CCK, which comprises about 50 percent of the CCK content in these tissues; and 4) in confirmation of the report by Meyer et al. (Science, 1982) DA innervation of the CP appears to contain little, if any, CCK.

In Dr. Chuang's group, Drs. Barbaccia (Visiting Fellow from Italy) and Brunello (Guest Worker from Italy) have studied the regulation and function of high affinity recognition sites for ^{3}H-imipramine and ^{3}H-mianserin. Using rat hippocampus, the synaptic locations of ^{3}H-imipramine and ^{3}H-mianserin binding sites and the role of these binding sites in the mediation of the therapeutic action of antidepressants were investigated. Degeneration of 5HT terminals elicited by 5,-7-dihydroxytryptamine (5,7-DHT) injections and lesion of the fimbria fornix led to a decrease in imipramine binding sites in the hippocampus but the Bmax of mianserin binding sites increased. Lesion with kainic acid decreased the Bmax of the binding sites for ^{3}H-mianserin without affecting the Bmax for ^{3}H-imipramine. These results indicate that the majority of the recognition sites for imipramine are located presynaptically while most of the recognition sites for ^{3}H-mianserin are present in the postsynaptic membrane facing 5HT innervation. Lesion of 5HT terminals with 5,7-DHT completely abolished the down regulation of beta-adrenergic receptors in the hippocampal and cortical membranes of rats treated chronically with imipramine or desipramine. Thus, an interneuronal system connecting the axons of 5HT and NE is operating during the imipramine-in

duced beta receptor down regulation. Following chronic treatment with imipramine or desipramine, the density of imipramine binding sites was decreased and the uptake of ^{3}H-5HT in the hippocampal slices was facilitated. No changes were detected in mianserin binding. We propose that this facilitation of uptake decreases the synaptic transmission in 5HT synapses and modifies the activity of the interneurons leading to the desensitization of beta-adrenergic receptors. Probably, this decrease determines a relief of the symptoms of depression.

We have also investigated the mechanisms of the mianserin-induced decrease in NE-sensitive adenylate cyclase. Lesion with 5,7-DHT failed to prevent the down regulation of NE sensitive adenylate cyclase present in brain cortical slices prepared from rats receiving repeated daily injections of mianserin, this suggests that mianserin fails to act on presynaptic terminals but exerts its effect after lesion of the serotonergic axons. It has been reported that mianserin labels $5HT_2$ recognition sites. We have found in rats treated chronically with mianserin that the number of binding sites for $5HT_2$ receptors was decreased whereas the binding sites for ^{3}H-mianserin was unaffected. These results led us to propose that mianserin labels a site related to but not identical to, $5HT_2$ receptors and that mianserin acts on this site to control serotonergic transmission in a negative manner that led to a persistent activation of $5HT_2$ recognition sites indirectly. To evaluate how the occupancy of imipramine recognition sites effects the serotonergic transmission, we studied how uptake of 5HT is changed in brain preparations of rats receiving daily injections of imipramine for 21 days. When the 5HT uptake is studied in synaptosomal preparations there is no change in uptake, but when the 5HT uptake is studied in slices of hippocampus, the uptake of 5HT is facilitated when the imipramine recognition sites are down-regulated. This finding prompted us to speculate that the imipramine recognition sites are activated by an endogenous effector which down regulates the uptake of 5HT. When the Bmax of these sites is decreased, there is a decrease of the negative control in the uptake of 5HT. Hence, the long term action of imipramine on 5HT transmission is a decrease in the synaptic transmission due to a more efficient reuptake. This, in turn, can generate an adaptive 5HT receptor response supersensitivity as reported by other investigators in their iontophoretic studies. Recent reports have suggested that deprenyl a specific inhibitor of MAO type B can relieve the symptoms of depression. Drs. Zsilla (Guest Worker from Hungary) and Barbaccia have shown that daily injections of deprenyl in doses that selectively inhibit MAO Type B, repeated for 21 days, cause a down regulation of NE-dependent adenylate cyclase, a decrease of the Bmax of beta-adrenergic receptor recognition sites and $5HT_2$ binding sites, and an increase in the Bmax of the imipramine binding sites. These results confirm the role of beta-adrenergic receptor down regulation in the profile of antidepressants, and indicate that presynaptic 5HT receptors play a role in the action of this drug. Probably, this antidepressive action is unrelated to the inhibition of MAO Type B, or to the formation of amphetamine for deprenyl because neither pargyline (a potent MAO inhibitor of Type A and B enzymes) nor amphetamine change the Bmax of imipramine binding.

Drs. Majewska (Visiting Fellow from Poland) and Chuang have initiated studies on the regulation of GABA receptor function in CNS. Crude synaptic membranes of rat brain, in addition to a low and high affinity Ca^{2+}- independent GABA binding, contain a Ca^{2+}- dependent high affinity GABA binding, termed $GABA_B$ binding sites. They investigated whether $GABA_A$ or $GABA_B$ sites, or both, are coupled to the recognition sites for benzodiazepines. It appears that $GABA_B$ binding sites are not coupled to the benzodiazepine recognition sites. The enhancement of ^{3}H-

GABA binding at 37° caused by diazepam (DIZ) was increased by 1 mM EGTA and inhibited by Ca^{2+}. When binding to $GABA_A$ and $GABA_B$ was differentiated by the presence of baclofen and THIP, respectively, binding to $GABA_A$ was enhanced by DIZ, but binding to $GABA_B$ was unaffected. Conversely, the enhancement of ^{3}H-flunitrazepam binding by GABA occurs only when $GABA_A$ receptor sites are free. The role of Ca^{2+} in the interactions of benzodiazepine and GABA recognition sites is now being investigated in great detail.

Dr. Chuang has continued to study the molecular mechanisms involved in the internalization of beta-adrenergic recognition sites in frog erythrocytes during desensitization induced by isoproterenol. We have previously found in frog erythrocytes, during isoproterenol-induced desensitization that there is an internalization of the beta-receptor recognition sites. Present experiments using various lysosomotropic drugs suggest that these soluble receptor sites are released from the endocytotic vesicles into cytoplasm due to partial hydrolysis by lysosomal enzymes. Moreover, subcellular fractionation of erythrocytes by Percoll gradient centrifugation revealed that at least one species of lysosomes retains beta-receptors and this receptor retention in lysosomes is enhanced when cells become desensitized. Treatment of the cells with various inhibitors of calmodulin, a calcium binding protein caused a time- and dose-dependent reduction in the extent of beta-receptor internalization and down regulation. These data are in line with the hypothesis that calmodulin is involved in regulating the clustering of beta-receptors in coated pits or vesicles which may be catalyzed by transglutaminase, a calcium dependent cross linking enzyme. Evidence is also presented that internalized beta receptor sites are recycled to the plasma membrane to restore the surface receptor density and functional sensitivity which are attenuated during desensitization.

Dr. Schwartz in collaboration with Dr. X. Breakefield (Yale University Medical School) has provided biochemical evidence that the genetically inherited neurological disease, familial dysautonomia(FD) is the result of a defect in nerve growth factor. The skin fibroblasts from patients with the disease contain NGF with only 10 percent of normal biological activity. Further chemical characterization of NGF in fibroblasts has proven extremely difficult because of its low content (0.001 % of total cell protein). Dr. Schwartz has prepared and purified mRNA from male mouse submaxillary gland. Injection of this mRNA into Xenopus oocytes results in the production of an immunoreative beta-NGF: incubation of the extract with the converting enzyme, gamma, results in an increase in both immuno- and bioactivity, suggesting that the mRNA codes for pro-beta-NGF. This mRNA has been inserted into a PBR-SV40 plasmid and converted into cDNA: plasmids have been cloned in E. coli and are being screened for the presence of DNA coding for NGF by means of the oocyte assay.

In order to understand better the biochemical and physiological effects of NGF deprivation, Dr. Schwartz has used an autoimmune animal model in which rats and guinea pigs are injected with mouse beta-NGF to which they develop antibodies. Animals exposed in utero to the anti-NGF resemble in many ways humans with FD. Loss of neurons occurs in both the sympathetic and sensory nervous systems. From previous work we know that certain peptides, such as Substance P, function as transmitters in sensory ganglia, and we have found large losses of these peptides in a number of peripheral tissues in the anti-NGF exposed animals. These losses can begin to explain some of the symptomatology of the dysautonomia, such as alterations in pain sensitivity and catecholamine secretion. Comparable studies on animals exposed to the anti-NGF only as adults have revealed that mature sen-

sory neurons require NGF for maintenance of transmitter function, although not for survival. In order to understand how NGF exerts these effects, we started experiments using the rat PC12 pheochromocytoma cell line, which has NGF receptors to investigate whether internalization of NGF with its receptor is required for biological activity and whether a membrane tyrosine phosphokinase is activated following the binding of NGF to its receptor.

Several types of cells in tissue culture have been used to study receptor pharmacology and receptor interactions at the molecular level. Dissociated cultures of rat anterior pituitary have provided a system to study dopamine D-2 receptors. Dr. Onali (Visiting Associate from Italy) has found that vasoactive intestinal peptide (VIP) stimulates both adenylate cyclase and prolactin secretion from the mammotrophs of the anterior pituitary. Dopamine can block both of these responses through an action at the D-2 receptor. Through experiments with GTP, GPP-NHP and cholera toxin, we conclude that the inhibitory coupling of the dopamine receptor with adenylate cyclase occurs at the level of the GTP binding protein, G/F. Since this enzyme is also a GTPase, we are currently investigating the role of GTPase in adenylate cyclase activity. Drs. Onali and Olianas (Visiting Associate from Italy) found that muscarinic receptors in the striatum are coupled to adenylate cyclase in an inhibitory manner. Occupation of these muscarinic receptors results in stimulation of a high affinity GTPase, and the pharmacology of the effects of acetylcholine on cyclase and GTPase is similar, suggesting that the activation of GTPase is coupled to the inhibition of cyclase by muscarinic agonists. The GH3 cell line provides a single cell population in which to study this question further. VIP stimulates both adenylate cyclase and prolactin secretion in these cells: acetylcholine blocks both of these responses at comparable doses. The role of GTPase in these will be of great interest to the area of receptor interaction.

Dr. Schwartz has studied the intracellular events triggered as a result of beta-catecholamine stimulation of adenylate cyclase using C6 glioma. Following a rise in cyclic AMP and activation of the protein kinase, the catalytic subunit of the kinase translocates into the nucleus and catalyzes the phosphorylation of specific acidic proteins. Induction of a specific form of cyclic AMP phosphodiesterase occurs 3-4 hours following catecholamine stimulation, and this induction is blocked by any drug that blocks one of the specific steps in this pathway, examples being vinblastine which prevents protein kinase translocation; or cordycepin which inhibits the stimulated nuclear phosphorylation. Inhibition of RNA polymerase II by incubation of the cells with alpha-amanitin also prevents PDE induction. From these studies we have learned a great deal about the molecular events triggered by receptor stimulation.

Group on Physical Methods (J.L. Meek, Ph.D., Group Leader)

The use of high pressure liquid chromatography (HPLC) in this laboratory has increased dramatically in the last few years as the power of the techniques for separation and detection of many compounds has become evident. The possibility of measuring peptides in tissue by HPLC has not been feasible because of problems of low sensitivity. Dr. Meek has been preparing and testing derivatizing reagents for peptides to allow for their measurement with increased sensitivity. Two promising reagents have been found which can improve detectability by 50-500-fold, while at the same time permitting high resolution separation of the peptides. Dr. Giorgi (Visiting Fellow from Argentina) has been using HPLC to measure rates of GABA accumulation in rat striatum following local injections of

GABA transaminase. He has examined the effects of the injection into the striatum of agonists and antagonists of the neurotransmitters of the striatum's main afferents and interneuronal pathways (glutamate, dopamine and acetylcholine).

Section on Biochemical Pharmacology (N.H.Neff, Ph.D., Chief)

The goal of the section is to provide new information about biological control mechanisms that modulate synaptic transmission. Emphasis has been placed on catecholamine and indoleamine transmitters, although there is significant interest in adenosine as a transmitter or modulator and on acetylcholine. Both presynaptic and postsynaptic biochemical events have been investigated with the aim of gaining insight into the mechanism of action of neuroactive substances.

Dopamine is a neurotransmitter for a subpopulation of retinal amacrine cells. Exposure of rats to light activates retinal tyrosine hydroxylase concomitant with an increase of dopamine synthesis. Activation of the enzyme is characterized by a decrease of the Km for the pteridine cofactor. Treatment with neuroleptic drugs results in an activation of the enzyme with a decrease of the Km for the pteridine cofactor as well. Dr. Cohen (Staff Fellow) has shown that tolerance develops to the ability of neuroleptics to activate tyrosine hydroxylase if the drugs are administered chronically. Chronic treatment with neuroleptic drugs does not alter the ability of exposure to light to activate the enzyme. Apparently, activation of retinal tyrosine hydroxylase by haloperidol and light occur by independent mechanisms.

Tyrosine hydroxylase in homogenates of retina from animals killed in the dark can be activated if incubated under protein phosphorylating conditions in the presence of cAMP. Dr. Iuvone (Emory University Medical Center) in collaboration with our laboratory, demonstrated that there are many similarities between activation of tyrosine hydroxylase in vivo by light and in vitro by protein phosphorylation. A summary of his findings suggest that activation of retinal tyrosine hydroxylase in vivo may be mediated by phosphorylation of the enzyme or by some effector molecule associated with the enzyme.

Drs. Economou (Guest Worker from Greece) and Cohen have identified norepinephrine and epinephrine in the rat retina. They represent about 10 percent of the total catecholamines present in the tissue. Moreover, these investigators observed that retinal homogenates were capable of methylating phenylethanolamine with S-adenosylmethionine-^{3}H as the methyl donor. The phenylethanolamine-N-methyltransferase inhibitor, SK&F 64139, blocked enzyme activity and there was a concomitant rise in norepinephrine and a fall in the epinephrine content of retina. There were no significant changes of enzyme activity in rats killed in the dark when compared with light. In contrast with enzyme activity, there was a significant rise of norepinephrine and a significant fall of epinephrine content in rats killed in the dark. Apparently, light and dark influence the catecholamine content of the retina but not phenylethanolamine-N-methyltransferase activity. This finding suggests that endogenous S-adenosylmethionine may be rate limiting for epinephrine formation in retina.

Drs. Lackovic (Visiting Associate from Youoslavia) and Relja (Guest Worker from Yougoslavia) prevously provided evidence for the presence of peripheral dopaminergic innervation of the urogenital system. In their current studies, they found that plasma membranes prepared from rat vas deferens contain specific binding sites for haloperidol. The binding sites have the characteristics of

a dopamine receptor. This observation generated speculation that some of the known effects of dopamine and dopaminergic drugs on sexual behavior might be mediated peripherally and not solely via the CNS as is usually assumed.

Drs. Economou and Potter (Guest Worker from Canada) found that the recently described serotonin-containing small intensely fluorescent (SIF) cells of superior cervical ganglion are, in part, modulated by preganglionic cholinergic neurons. For example, administration of the muscarinic agonists carbachol or oxotremorine increased the content of serotonin, and the increase induced by oxotremorine was blocked by atropine. Treatment with atropine alone or decentralization of the ganglion lowers the content of serotonin. Reserpine, p-chlorophenylalanine or fluoxetine treatment reduces the content of serotonin in the ganglion, suggesting that the SIF cell system has properties similar to serotonergic neurons of brain. Apparently, serotonin-containing SIF cells of the rat superior cervical ganglion participate in local circuit modulation of ganglionic transmission by receiving preganglionic information via muscarinic receptors.

All serotonin containing nerve fibers in the cord have been assumed to originate in the midline raphe nuclei complex. In the brain, serotonin and Substance P are often found together in the same neuron. Dr. Economou investigated whether serotonin might be found in the sensory ganglion which is known to contain substance P. She evaluated the possibility that serotonin-containing interneurons might be present in the spinal cord. By chemical analysis she found the content of serotonin to be higher in the lumbar portion of the cord, the ventral horns to contain more serotonin than the dorsal horns, and the ventral and dorsal roots to contain about the same content of serotonin. Ten days after mid thoracic transection of the cord there was about a 95 percent decline of serotonin and after rhizotomy there was no change in the serotonin content in the cord. Of particular interest was the observation that there was a decline of serotonin in the ventral root but not in the dorsal root below a spinal cord transection, suggesting that serotonin axons might leave the cord via ventral roots. Drs. Panula and Economou have initiated immunohistochemical studies of the cord after transection, using an antibody to serotonin. Transection decreased immunoreactivity throughout the cord except in layer X. Thus far, only scattered mast cells have been found in the nerve roots and sensory ganglia. From these preliminary studies it appears that there may be serotonin interneurons in layer X whose efferent fibers leave the spinal cord via the ventral roots.

Adenosine has been proposed as a neurotransmitter or modulator because it has biochemical, electrophysiological and behavioral action in animals. Dr. Wojcik (PRAT Fellow) developed a simple HPLC method to assay adenosine in brain and found that adenosine was uniformly distributed in the brain of rats killed by focussed microwave radiation to the head. In contrast, if the animals were killed by decapitation there was an uneven distribution of adenosine, with the content in the striatum increasing by as much as 50-fold. Interestingly, this was the only brain structure where an adenosine-stimulated adenylate cyclase system could be detected. From the results of experiments with surgical brain lesions or the injection of neurotoxins, Dr. Wojcik concluded that striatum contains intrinsic adenosine neurons and receptors.

There are two plasma membrane adenosine receptors in neuronal tissue, a high affinity receptor which inhibits adenylate cyclase, termed A_1 and a low affinity receptor associated with the activation of adenylate cyclase, termed A_2 . Dr.

Wojcik found that the A_2 receptor appears to be located primarily in striatum on neurons that are destroyed by treatment with kainic acid. In contrast to A_2 the presence of A_1 receptors in neurologically mutant strains of mice with specific lesion in cerebellum, Dr. Wojcik concluded that A_1 receptors are associated with Prukinje cell dendrites and/or granule cells in cerebellum.

Dr. Potter has developed a rapid, simple method for acetylcholine and choline. The method is based on the separation of acetylcholine and choline by reverse phase HPLC and by mixing the effluent with acetylcholinesterase and choline oxidase. Choline oxidase converts choline to betaine and hydrogen peroxide. Production of hydrogen peroxide is continuously monitored with an electrochemical detector. The assay takes about 10 minutes and the sensitivity for the detection of choline and acetylcholine is 1 and 2 pmol, respectively. Because no prior derivatization or separation of acetylcholine is required, sample preparation is rapid. The method is simple and reproducible and offers excellent sensitivity and specificity.

Section on Molecular Pharmacodynamics (D.L. Cheney, Ph.D., Chief)

Two major advancements have occurred in the section during FY 1982, which include the introduction of histological studies under the direction of Dr. Panula and the introduction of behavioral studies conducted by Dr. Blaker (Staff Fellow) to complement the biochemical studies on transmitter dynamics currently under way in the section. During the past several years, while studying the regulation of acetylcholine turnover, we had identified biochemically, numerous transsynaptic interactions within the septum and hippocampus involving a number of putative neurotransmitters. Unfortunately, histological evidence to locate and understand the functional significance of these interactions was lacking. Now with the addition of histochemistry and behavior to the section, we hope to answer two important questions, 1) can the interactions between the neuromodulators that have been identified biochemically also be shown to exist histologically?; and 2) are these interactions functionally relevant in identifiable behavior paradigms? Although these studies are at their inception, early results are exciting and suggest that the answers to these questions may be forthcoming.

GABA agonists injected into the septum have been shown to inhibit the cholinergic activity in neurons projecting to the hippocampus. Drs. Panula and Revuelta (Visiting Associate from the Philippines) have shown that glutamate decarboxylase, a specific marker of GABAergic neurons in the central nervous system is widely distributed in the septum of the rat and that neuroanatomical interactions between the GABAergic interneurons and cholinergic cell bodies might possibly occur. Further confirmation, however, must wait for electron microscopic studies. Glutamate decarboxylase positive cell bodies are most numerous in the medial septal nucleus and the nucleus of the diagonal band with only a few scattered cell bodies in the lateral septum. Dense networks of immunoreactive fibers and terminals have been found in all parts of the lateral septal nucleus. Drs. Panula and Revuelta have demonstrated that Met-enkephalin and beta-endorphin which inhibit cholinergic dynamics when injected into the septum have quite different pattterns of distribution within the septal complex. Met-enkephalin immunoreactive fibers and terminals are seen consistently in the intermediate part of the lateral septal nucleus. In colchicine-injected rats numerous met-enkephalin immunoreactive cell bodies have been found in the dorsal, intermediate, and ventral parts of the lateral septal nucleus. Fewer cells have been found in the medial nucleus and the diagonal band. No cell bodies exhibiting beta-endor-

phin-like immunoreactivity have been found in the septal complex but the basal parts of both the lateral and medial septum have been shown to contain beta-endorphin immunoreactive fibers. These results are consistent with the hypothesis that the enkephalin-containing neuronal system in the septum consists of septal interneurons whereas the beta-endorphin system comes from the hypothalamus.

Other studies originated by Dr. Panula include localization of novel peptides in the central and peripheral nervous system to identify possible interrelationships between neuronal systems and previously characterized pathways and target organs. Bombesin and substance P have been found in the same cell groups in the nucleus tractus solitarii, dorsal parabrachial nucleus and dorsolateral tegmental nucleus. Bombesin, but not substance P, has been found in the paraventricular nucleus. In the periphery, bombesin-like immunoreactivity has been found in the spinal sensory ganglia, adrenal medulla, and nerve fibers innervating the lung. Met^5-enkephalin-Arg^6-Phe^7-like immunoreactivity has been found in the same areas of the brain as Met-enkephalin.

A variety of pharmacological manipulations of the medial septum in the rat brain have been shown to result in changes in the activity of the septal hippocampal cholinergic pathway. Furthermore, studies have been done on the involvement of these limbic structures on various behaviors. Impairment of response inhibition can be measured by recording sustained responding during extinction. This phenomenon can be induced by septal hippocampal, or fornical lesions, or by administration of anticholinergics to the hippocampus. Dr. Blaker has injected the GABA agonist, muscimol, into the septum and has studied the dynamics of the cholinergic system to the hippocampus as a function of the extinction of a food reinforced lever press response. He has found that doses of muscimol which decrease the turnover rate of acetylcholine also increase the response rate during extinction. He has concluded that a muscimol-induced decrease in hippocampal turnover rate of acetylcholine is accompanied by interference with extinction, but that operantly induced differences in this behavior are not accompanied by large changes in the turnover rate of acetylcholine located in the hippocampus.

In other studies in the section, Dr. Wroblewski (Visiting Fellow from Poland) has begun to investigate the pharmacological regulation of the glutamatergic pathways in the rat brain. In order to develop a method to measure glutamate turnover one approach used by Dr. Wroblewski was to inject deuterium labeled L-glutamate intracerebroventricularly and measure the incorporation of the label with time into various structures of the rat brain. The slope of the exponential decline of the incorporated isotopic label would be an indication of the functional dynamics of the glutamatergic system. Dr. Wroblewski has shown that within 5 min. after the injection of L-glutamate, the maximal concentration of labeled glutamate is reached in all brain parts studied and the decay is exponential in all areas studied. The amount of label incorporated is three-fold higher in the septum than in the cortex, striatum, or hippocampus. Further investigation will determine whether the kinetics of the decay of the labeled glutamate are modified when glutamatergic pathways are stimulated or lesioned.

In a collaborative study with Dr. J. Hong (Research Triangle Park, N.C), Dr. Gandolfi (Guest Worker from Italy) is studying the neurotransmitter mechanisms which cause chlordecone (kepone) toxicity. Kepone has been found in man to cause neurotoxicity, including tremors, headaches, abnormal elevation of CSF pressure, mental symptoms, and visual disturbances. The compound accumulates in the body and appears to affect primarily the neuronal system, the reproductive

system and the liver. Dr. Gandolfi has shown that in adult male rats receiving a single injection of kepone there is decreased GABA binding in cerebellar and hippocampal membranes and a decreased mianserin binding in hippocampal membranes. Flunitrazepam and imipramine binding are unaffected in any area studied. Because of the suggested involvement of GABA receptors and serotonergic receptors, GABA mass spectrometric and serotonin high performance liquid chromatographic turnover studes are in progress.

Annual Report of the Research Services Branch

National Institute of Mental Health

National Institute of Neurological and Communicative Disorders and Stroke

October 1, 1981 - September 30, 1982

The Research Services Branch (RSB) provides broad technical support for the Intramural Research Programs of NIMH and NINCDS through (1) research and development in advanced biomedical instrumentation techniques and systems; (2) evaluation, specification and management of computer systems; (3) direction of a program of laboratory animal medicine and care (NIMH only); and (4) provision of other technical services in support of the research program.

The Branch is comprised of two sections:

Section on Instrumentation and Computers provides technical support for investigators by (1) assessing the instrumentation and computer needs of the investigator; (2) designing, developing and constructing special-purpose electronic and mechanical instrumentation and systems not commercially available; and (3) designing, specifying and managing laboratory computer systems for data acquisition and processing.

Section on Laboratory Animal Medicine and Care provides professional advice and assistance to intramural scientists on all aspects of animal health, medical care, and testing and surgical protocols; manages a central primate holding facility; and conducts a program of laboratory animal care.

Additional services provided by the Branch include consultation on measurement techniques, signal processing, noise and electro-magnetic interference in data measurement systems, and equipment purchases. Several formal and informal courses for investigators are taught by Branch personnel; topics include electrical circuit theory, operational amplifier applications, digital logic design, and computer applications.

Due to manpower limitations and economic considerations, the Branch is unable to provide the following services: repair of commercial instruments, duplication of off-the-shelf commercially available equipment, and fabrication of non-instrument items (shelves, bookcases, etc.).

When an investigator requires the services of the Branch, he first meets with the Branch Chief and other personnel as needed to discuss his requirements. On the basis of this meeting, a decision is made as to whether RSB will take on the project. If a commercially produced instrument will satisfy the investigator's requirements, he is advised to purchase it. If custom instrumentation is needed, RSB will accept the project unless we lack the appropriate expertise, or our current work backlog is excessive. In these cases the project may be contracted to a private firm, or the investigator may be directed to the Biomedical Engineering and Instrumentation Branch (BEIB).

When the Branch Chief or the Assistant to the Chief agree to accept a project, the investigator submits a standard work request form (available from RSB), signed by his Lab Chief. This form will state the nature of the instrument or service requested, and will contain as many details and specifications as the investigator con provide.

The project is then assigned to an engineer, who will confer with the investigator to formulate a set of engineering specifications and a timetable and cost estimate for the project. The RSB does not charge for services, but the investigator will be billed for the cost of the components used. Upon delivery of the completed instrument, a memo is sent to the investigator listing the component costs and asking permission to have the Administrative Officer transfer funds from his CAN to the Branch's CAN.

SECTION ON INSTRUMENTATION AND COMPUTERS

INSTRUMENTATION

The Branch has a staff of six engineers and six technicians to design, develop, and fabricate electronic and mechanical instruments. The major effort is in the production of electronic instruments for basic neurophysiological research, and for clinical studies involving affective disorders. The following are brief descriptions of representative projects, chosen from a total of 310 projects completed this year.

(1) Patient Activity Monitoring System. The Section has continued to develop the Patient Activity Monitor (PAM) and the support hardware and software which forms the system. The standard PAM, which provides 64 hours of data at 15 minute intervals, was redesigned to use batteries which have 18 months capacity. The software has been expanded, and a provision has been made to acquire and store data from a commercially available temperature monitor which utilizes the same data storage principal as the PAM.

A program was written to allow activity or temperature data to be stored in continuous files of unlimited length; this simplifies long-term data analysis. Numerous other programs, including automatic sleep recognition and various graphical data presentation techniques, were added this year. An extensive users guide to the PAM software was written.

The major hardware advance this year has been the development of a PAM to replace the hybrid monitor. The hybrid, which stores over 10 days of data and is much smaller than the standard PAM, was based on the technology of connecting integrated circuit dies to thick-film printed substrates by microminiature wire bonds. These hybrid PAMs were fabricated by a private contractor. Unfortunately, they proved to be very failure prone, and the devices cannot be repaired. We have abandoned this technology, and are completing development of a PAM with all the characteristics of the hybrid, but much more reliable and less expensive. It is easily fabricated, using pre-tested parts, and can be repaired if necessary. It will become our standard device, and will eventually replace all the older PAMs.

The PAM is being evaluated for use in determining sleep stages, without taking sleep EEG recordings. PAMs are placed on the head, trunk, and wrist or ankle; algorithms are being developed to determine sleep stage as a function of relative activity at the three sites. If successful, this technique would greatly expand the possibilities for outpatient sleep research.

(2) EEG Amplifier System. A 32-channel EEG amplifier system was designed for use in several ongoing research projects involving topographic brain mapping. The design incorporates several new electrical components which permit construction of a compact, low cost-per-channel unit. The system consists of a preamplifier, located next to the subject, joined to a main amplifier by a flat cable. The signal gain in the preamplifier is 10,000 and, in the main amplifier, 30, for an overall gain of 30,000. An important part of the amplifier design is the filter section. The filters were designed to prevent aliasing errors when the signal is digitized; to eliminate any phase distortion in the passband region that would interfere with time-series average evoked response analysis; and to have a good step response, to minimize "ringing" resulting from stimulus artifacts. The design also includes a sample and hold module on each channel to prevent any "skewing" errors associated with A/D conversion.

(3) Computer-Controlled Trapezoid Generator. A torque motor position control system was previously developed by RSB for research on the mechanisms which produce the tremor of Parkinson's disease and also for general neuromuscular research. A signal with a trapezoid timecourse which could be synchronized with the data collection computer was thought to be a very useful command input to the torque motor system. To satisfy this need, a precision, computer-controlled trapezoid generator has been developed. A PDP-11 minicomputer loads the trapezoid parameters (up time, hold time, down time, and amplitude and polarity) into the generator via a parallel digital interface. A fifth parameter from the computer starts the trapezoid waveform and sets the duration of the control signals for the torque motor system. By allowing synchronization between stimulus and computer data acquisition, this trapezoid generator has greatly facilitated use of the position control system.

(4) Tissue Culture Voltage Clamp System. A voltage clamp system has been developed for investigating the membrane properties of electrically and chemically excitable tissue culture cells. This low voltage system was designed for use with two high impedance glass microelectrodes to clamp slow-to-medium speed neuronal voltage changes but not action potentials. Two identical headstage amplifiers are provided so that after electrode placement in the cell, either electrode may be used to measure the membrane potential. These headstage amplifiers also may be used as constant current sources for current clamping experiments. A dual sensitivity/speed virtual ground current monitor is also provided.

(5) Discriminator and Iontophoresis Systems. The RSB amplitude/time window discriminator system continues to be an important signal processing tool in neurophysiological studies. The versatility of this system has been increased and the design simplified by implementing the circuitry with CMOS logic. Six of these new discriminator systems were completed this year, and two modified units for processing post-synaptic potentials are under construction. Although micropressure ejection of drugs from multibarreled pipettes has become, in many cases, the preferred method of drug application, the use of microiontophoresis is still widely used. Four of the RSB 5-channel iontophoresis systems are presently nearing completion and will be used in neuropharmacological studies in the IRP.

(6) Data Acquisition System for Isolation Rooms. Two isolation rooms are being designed to permit the study of biological rhythms and the cyclic nature of certain mental illnesses. Each room will be occupied by one human subject who will be isolated from all time cues. RSB is designing a computerized data acquisition system for these rooms so that activity and temperature data can be periodically recorded. Recording mood self-ratings and limited subject-staff communications will be provided by special touch-input CRT terminals.

(7) Resistance Monitor and Shutter Controller. In order to view a freeze-fractured sample of tissue with an electron microscope with greater resolution, a thin layer of metal is first deposited on the sample. The resulting resolution will be dependent on the amount of metal deposited and the length of exposure time of the sample to the heat of the ion gun. A controller has been developed that monitors the amount of metal being emitted by the ion gun by measurement of resistance changes as metal is being deposited between two terminals separated by a fixed length and width of non-conducting fiberglass board. A shutter, which can be opened and closed at variable resistance limits, is used to control the amount of material deposited and to make the exposure time of the sample to the heat of the ion gun as short as possible.

(8) Visual Evoked Response Stimulus System. A visual evoked response stimulus system has been built, that will randomly select one of eight 35mm slide images and project it on to a 35cm x 50cm opaque screen. The projection system uses a very fast electromechanical shutter (2.3 msec. opening time) for a fast rise time in presenting the image. The slides are mounted on a circular disc, which is rotated by a direct-drive stepper motor. The maximum random access time for any slide is 125 msec. The stepper motor is controlled by a special purpose processor that can be linked to either a computer or a terminal through a standard RS232 serial interface.

(9) Neuro PET Scanner Chair and Gantry Controller. A controller is being designed for the Neuro PET Scanner which was developed by NINCDS in conjunction with BEIB. This controller will facilitate the positioning of the patient's head into the scanner through control of an electromechanical chair. This device will insert the patient's head a fixed distance into the aperture of the scanner from a predetermined setup position. The controller will provide a digital readout of the patient's position and will include various safety stops to prevent collision of the chair and the gantry.

(10) Programmable Infusion Pump. A microprocessor-based instrument was developed to control a motor driven syringe platform (infusion pump). The pump is used to maintain a constant arterial concentration of infused substances during absorption studies with laboratory animals. The pump delivers an initial bolus followed by an exponentially decreasing infusion pumping rate. A calibration mode is available for generating syringe and motor calibration coefficients. Initially the instrument prompts the operator for pumping schedule. An on-line pumping schedule listing is available or a pre-pumping listing of the programmed schedule may be requested. The listing provides the pumping rate/volume per delta time for the infusion schedule and the total volume delivered.

(11) Four-Arm Radial Rat Maze. An elevated multi-level 4-arm radial rat maze is being constructed and instrumented to assess the effects of neuropeptides on learning, memory, and perception in experimental animals. Audible and/or visual cues will be presented at the end of a randomly selected arm. The path

of the animal is monitored by detectors located at selected positions throughout the maze. When an animal traverses the proper path to the cues, a programmable liquid reinforcement will be dispensed. At the end of the testing period, statistical data will be printed regarding the animal's performance. An 8-bit microprocessor single board computer is used to monitor and control the maze and perform the necessary statistical calculations.

COMPUTERS

The Section on Instrumentation and Computers continues to support the use of the computer as a laboratory instrument. Small computers are used in the individual laboratories for on-line, real-time interaction, process control and data acquisition. The Section maintains support computers in Buildings 10 and 36. These systems provide means for program preparation, bulk storage, printing and plotting, and mathematical and statistical processing. Experimental data may be transmitted from the laboratory computers, via these systems, to the DCRT facilities for further processing. The support computers also serve to develop prototype systems and to test the feasibility of the use of a computer in specific laboratory applications. The latter capability allows an investigator, once he determines that the computer will do the job, to purchase an efficient system at minimal cost. The Section also maintains an image processing system, described below.

The Section provides software support for the individual investigators. A library of procedures has been developed that is tailored to the needs of the Intramural Program. Individual training is available for investigators with no prior experience in using or programming the computer. Computer specialists are available for consultation in all areas of computer use, programming, interfacing, real-time applications, time series analysis, data presentation, systems configuration and computer procurement. Although the Section does not provide an applications programming service, systems have been developed in collaboration with individual laboratories. Examples are included in the list of computer projects.

Program maintenance is an important function of the Section. Programs used in a real-time interactive laboratory research environment often produce new information which calls for modification of the program before the next experiment. In addition to the software library and research related projects developed by the Section, much work is caused by the turnover of scientific and support personnel. Many systems developed by these persons prove useful to the laboratory. After they leave, maintenance of such systems becomes the responsibility of the Section. Structured programming techniques and standardization on PASCAL have enabled the Section to provide these services without an increase in personnel. There are currently more than 50 minicomputers in the Intramural Program.

The Section also maintains a microprocessor development system for software and hardware development of microprocessor-based instrumentation at both the chip level and the single board computer level. The system currently supports three common microprocessors, one 16-bit processor, and two 8-bit processors. Various utility programs and two high level language compilers are available (FORTRAN and PLM) for application programming.

The support computer in Bldg. 36 was upgraded this year, and with the acquisition of two laboratory systems for program development, much of the burden on this facility has been somewhat relieved. However, increasingly sophisticated mathematical algorithms are being developed in the areas of image processing, cell membrane analysis, and digital signal processing. These techniques require an increasing amount of processor time, and the existing single user systems are not the most cost effective method of handling these problems.

A Digital Equipment Corp. VAX-750 32-bit computer has been installed in Bldg. 36. Space for this facility is furnished by the Laboratory of Cerebral Metabolism. This computer processes mathematical data more efficently than any of the existing 16-bit computers and has a time shared, virtual memory operating system. It has a compatibility mode in which programs written on the existing computers will run with little or no modification. Programs may be written and compiled on this system to be run on the laboratory computers. The two existing image processing systems will be linked directly to this computer, via a high-speed communication linkage. Future plans call for connecting laboratory computers to the facility and develop a true distributed network. This will provide increased capability for the laboratory satellite, at less cost to the user.

IMAGE PROCESSING SYSTEM

The Section on Instrumentation and Computers maintains a general purpose image processing system. This system consists of a high-speed rotating drum scanner, an image array processor and display, and a PDP-11/60 computer. The drum scanner can digitize transparencies up to 10x10 inches with spatial resolution of 12.5 microns. The image array processor can simultaneously store, display, and manipulate up to three 512x512 digitized images. Images may be compared, superimposed, translated, zoomed or color coded at video rates. Images to be processed may be obtained by scanning autoradiographs, x-ray film, or photographic negatives, or by using previously digitized images generated by CAT or ECAT scanners. A camera station has been added this year.

An interactive, menu driven, software system provides an extensive and expandable repertoire of basic image processing and input/output functions. Special purpose functions can be developed to meet specific user requirements. The facility is useful for numerous applications involving evaluation and quantification of biomedical images. Two applications, however, are primary: analysis of two-dimensional electrophoresis gels and analysis of autoradiographs of brain or tissue sections.

The autoradiographs are used for measurements of glucose utilization in brain tissue using the Sokoloff deoxyglucose method of glucose substitution. Analysis of the autoradiographs involves displaying the digitized image on a TV monitor and outlining areas of interest. The average optical density is then computed and automatically converted to glucose utilization. Glucose utilization of brain regions as small as 100 microns in diameter can be computed. A color coded glucose utilization map may also be produced.

Measurement of amino acid concentrations can be made using two-dimensional electrophoreses gels. The gels, which have been prepared by the appropriate stain and fixer, are photographed; or if radioisotopes are used, an autoradiograph is obtained. The film is scanned and digitized into an array of optical

density within a defined boundary. A test gel may be compared with a standard gel using the image array processor to determine the presence or absence of a particular substance.

Additional examples of computer projects include:

(1) Fine Motor Control Evaluation Project. Programs have been developed for evaluating fine motor control movements in Parkinson patients using a peripheral device called the Bit-Pad I (Summographics Corp.). It consists of a magnetostrictive surface sensitive to the position of a pen-like stylus. The device transmits the position of the stylus through a standard computer interface. The program determines, over a series of trials, how well the subject can move the stylus through a series of positions on a pattern, in a connect-the-dots fashion. Each trial consists of at least five successive repetitions. Evaluation is based upon speed and accuracy. The reaction time of the subject is measured at the start of each trial; it is thought that this can be related to the number of positions in the pattern that the subject must subsequently traverse. The test should be a sensitive indicator of the amount of fine-motor dysfunction in Parkinson patients.

(2) Cell Culture Analysis. This system is designed to provide an on-line analysis of tissue culture neurons. The first phase, to study the excitatory or inhibitory post-synaptic potentials of these cells, has been completed. A unique feature of this system is the on-line control of artifacts introduced by the measurement system and the properties of tissues in culture and to control the threshold levels and amplification level as the experiment is in progress. Visual displays of amplitude, integral and latency are available, as well as averaged evoked response. In addition, on-line monitoring of post-synaptic potentials elicited by stimuli presented in pairs or in trains of pulses are available. The system also studies spontaneously occurring miniature potentials. This system is being extended to allow analysis of the cells by other techniques such as voltage clamping and the iontophoretic injection of neuroactive substances on the surface of the cell.

(3) Neurophysiological Data Analysis System. This system was initially developed for the Laboratory of Neurophysiology, NIMH, and has found widespread use. It is a versatile system for the collection and analysis of neurophysiological data, such as cortical unit events, lever position, EMG, etc., with behavorial events, and allows the presentation of this data in its relation to any time locked variable. The data are displayed as rasters and histograms of the neural events, centered on behavioral criteria, with the ability to mark selected events, and also the analog sweeps associated with these trials. Extensions are being made to this system to enhance its utility; these include the ability to select groups of trials within a unit, the selective deletion of sweeps, and the shifting of individual rasters in time. In addition, the individual trials may be sorted on a number of variables included in the data. A time window may be selected and a sort made on the pulse count (neural events) or selected criteria from the analog data such as the integral, slope, maximum amplitude or the latency to the first derivative.

SECTION ON LABORATORY ANIMAL MEDICINE AND CARE

The Section on Laboratory Animal Medicine and Care is staffed by a veterinarian, laboratory animal technicians and animal caretakers who provide daily care and maintenance of laboratory research animals. Services provided by the Section include:

(1) Management of the Central Primate Facility. The Central Primate Facility has a capacity for approximately forty primates. At the present time, three investigators have research projects in progress. Support is provided through assistance in preparing surgical models, insertion of catheters and maintaining animals on special diets and feeding routines. The Section has also developed the capability for bacteriological culturing and identification, pH and blood gases, and non-invasive blood pressure monitoring.

(2) Tuberculosis Testing Program. All primates, including those located at St. Elizabeth's Hospital are tuberculosis tested every three months.

(3) Treatment of sick and post-operative animals. Assistance in this area has greatly increased over the last year primarily due to an outbreak of roto and adenovirus infections in the primate nursery. In collaboration with the Virology Section of NIAID, the viruses were isolated, identified and sufficient control measures have been put into effect.

(4) Assistance in animal procurement when needed. The NIH Primate Quarantine Unit has been unable to supply the NIMH with sufficient numbers of rhesus monkeys needed due to lack of animals from the breeding colonies. This Section was able to purchase twenty animals from Litton Bionetics for use by NIMH investigators. Efforts are continuing to find additional animals.

(5) Providing day to day care and maintenance of research animals. Care and maintenance of research animals are provided seven days a week in all areas. The supervisor of the Section provides day to day contact with personnel in all work areas and coordinates weekend and holiday schedules. Night coverage of the primate nursery continues to work very smoothly with two part-time persons covering on alternating nights. Also, these two persons administer night medications to animals on treatment.

(6) Animal Facilities. The primate holding facilities in the ACRF have been completed and are ready to be occupied. The only major discrepancy in these facilities is the lack of proper air changes. Efforts are being made through the NIH Animal Care Committee and the Division of Safety to correct this deficiency. The animal care facilities at St. Elizabeth's Hospital are being upgraded. An incinerator will be installed this year and a contract for installation of an elevator to the second floor cagewasher is being initiated. Plans for renovation of Building 9 and its animal facilities are progressing with final plans to be completed by September 30, 1982. This renovation should greatly improve the ventilation and drainage systems for the building. Although the animal holding facilities at St. Elizabeth's Hospital and Poolesville are not part of the Section due to logistic difficulties, the veterinarian is responsible for the quality of animal care delivered. Consultant visits are made periodically to assist in care and maintenance of research animals and to take care of health problems that may exist.

ENGINEERING, COMPUTER AND FABRICATION SERVICES

This table shows the distribution of the Branch's workload among the various laboratories and branches.

LABORATORY OR BRANCH	HOURS	PERCENT
Clinical Science, NIMH	2543	9.49
Neurophysiology, NINCDS	2511	9.37
Neurophysiology, NIMH	2475	9.23
Biological Psychiatry, NIMH	2428	9.06
Clinical Psychobiology, NIMH	1902	7.09
Cerebral Metabolism, NIMH	1738	6.48
General and Comparative Biochemistry, NIMH	1541	5.75
Biophysics, NINCDS	1403	5.23
Neuropathology and Neuroanatomical Sciences, NINCDS	1312	4.89
Molecular Biology, NINCDS	1096	4.09
Neurochemistry, NINCDS	1012	3.77
Surgical Neurology, NINCDS	987	3.68
Experimental Therapeutics, NINCDS	710	2.65
Neuropsychology, NIMH	594	2.22
Psychology and Psychopathology, NIMH	509	1.90
Adult Psychiatry, NIMH	438	1.63
Molecular Genetics, NINCDS	359	1.34
Infectious Diseases, NINCDS	270	1.01
Neurochemistry, NIMH	175	.65
Brain Evolution and Behavior, NIMH	159	.59
Neuroimmunology, NINCDS	158	.59
Clinical Neurosciences, NINCDS	150	.56
Neural Control, NINCDS	121	.45
Central Nervous System Studies, NINCDS	101	.38
Other Laboratories and Branches, NIMH	98	.37
NIMH (Total)	14,600	54.45
NINCDS (Total)	10,190	38.01
NICHD (Total)*	2,019	7.54
	26,809	100.00

*NICHD loans the Branch one position, and is thus entitled to 1700 hours of service.

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